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CLINICAL LABORATORY THERAPEUTIC

BY

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PREFACE TO THE TWELFTH EDITION

In preparing this new edition we have taken the opportunity of thoroughly revising our book, and of adding about one hundred pages of new material. As the size of the book is little changed, it has been necessary to omit certain sections, including the chapters dealing with the kidneys and diphtheria. In Chapter I, now entitled Chemotherapy, the mode of action of the sulphonamide drugs has been rewritten, a note included on sulphamerazine, and a new section added on synthetic anti-malarial drugs. The penicillin Chapter is new, and in it penicillin is discussed both from the laboratory and the clinical aspects. This Chapter also contains an article on other antibiotic substances such as streptomycin, streptothricin and tyrothricin. Many alterations have been made in the Vitamin Chapter. The descriptions of the vitamin B complex and of vitamin K have been largely rewritten, and notes added on biotin, folic acid and dicoumarol.

New articles include those dealing with thiouracil, primary atypical pneumonia, infective heptatitis, homologous serum jaundice, bone marrow transfusion, and the use of thiocyanates in the treatment of high blood pressure. In the Sex Hormone Chapter the sections dealing with the urinary hormones for diagnostic purposes and the assay of the neutral 17 keto-steroids have been rewritten, and a fresh section prepared discussing the treatment of malignant disease by synthetic œstrogens. The last Chapter dealing with the methods has been rewritten and new descriptions added, which include such procedures as the estimation of the acid and alkaline phosphatase, the estimation of abnormal blood pigments, carboxyhæmoglobin, sulphæmoglobin and methæmoglobin, the estimation of thiocyanates in the blood and of the neutral 17 keto-steroids in the urine.

We wish to thank Messrs. Allen and Hanburys for permission to reproduce Fig. 38 showing the bone marrow transfusion needles, and Messrs. Adam Hilger Ltd. for Fig. 40 illustrating the Photoelectric Absorptionmeter.

We are grateful to Dr. A. E. Kellie for help in the biochemical sections.

Our publishers have kindly allowed us to make extensive changes in this new edition, and we are very grateful to them for their continued help.

G. E. B.
E. C. D.

LONDON.

PREFACE TO THE FIRST EDITION

DURING the last decade changes have taken place in medicine, especially in the routine methods adopted in the clinical and laboratory investigation of disease, and also in certain forms of treatment.

This book has been compiled with the following objects: to assist practitioners who have not had the opportunity of recent post-graduate study, to familiarise themselves with some of these advances; to provide a reference book for those who are working for the higher examinations in medicine; to give candidates studying for the primary examination for the Fellowship of the Royal College of Surgeons an account of the application of physiological and biochemical principles to medicine. It is also hoped that it will prove of assistance to the laboratory worker in that the recent chemical methods are dealt with in detail. It should also form a link between the wards of a hospital and the laboratories, giving fuller details of methods which are alluded to in medical textbooks, but often omitted from the handbooks on clinical methods. The recent work of American and Continental authorities has also been incorporated.

One of the chief difficulties has been to decide the actual scope of the contents, which of necessity encroach upon the domains of theoretical medicine, therapeutics, biochemistry and bacteriology. The guiding principle has been to confine the subject-matter to a description of such methods of diagnosis and treatment as are

used for medical patients in a general hospital, and which can be justly termed " recent advances in medicine."

Great care has been taken to give a workable description of each procedure, all techniques described have been performed personally by one or other of us, and the accounts are taken from our notebooks. Although these may differ slightly from the original descriptions, the methods described have been used by us as a routine for some time, and have given very satisfactory results. We have attempted to state the value of the results obtained by the various tests.

The authors wish to express their indebtedness to the numerous writers whose works have been consulted, and an endeavour has been made to acknowledge them by the list of references.

We have pleasure in thanking Sir Thomas Lewis for permission to reproduce the electrocardiograms taken from his book entitled " Clinical Electrocardiography." Dr. D. E. Bedford has supplied the polygraph tracings, and has also read through the proofs of the chapter dealing with the heart. We are grateful for his valuable suggestions and help. Further, we wish to express our thanks for the loan of certain blocks : to Messrs. Hawksley & Sons for the one illustrating the Jacquet polygraph ; to Messrs. Down Bros. for those illustrating the pneumothorax needles ; and to Professor Harris for the diagram of the electrocardiograph from Anrep and Harris' " Practical Physiology."

In conclusion we wish to acknowledge the unfailing help which we have received from the publishers of this volume.

G. E. BEAUMONT
E. C. DODDS

London, 1924.

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RECENT ADVANCES IN MEDICINE

CHAPTER I

CHEMOTHERAPY

The inception and development of chemotherapy belong exclusively to the twentieth century. Prior to 1900 very few purely synthetic compounds had been introduced into medicine, most of the remedies used being extracted from plant and animal sources. That purely synthetic compounds might be found which, whilst showing selective toxicity against the protoplasm of pathogenic organisms, would be tolerated by the tissues of the host organism was first conceived by Paul Ehrlich. There is little doubt that his faith in such a possibility carried him over the numerous setbacks which led to the final success of his classical work on synthetic arsenical drugs.

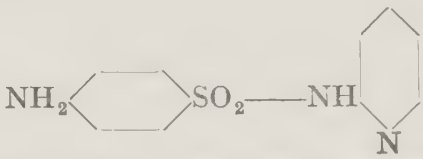
In this work Ehrlich knew something of the relentless search for high potency below the threshold of the minimum lethal dose. In almost fifty years of development others have shared in the vicissitudes of this struggle and, if the team is stronger, the difficulties are no less real. The introduction of the sulphonamides during the last decade has dwarfed previous discoveries in the field.

Sulphonamide Drugs

In 1935 Domagk (1) demonstrated that a new azo dyestuff, sulphonamidodiaminoazo-benzene (prontosil rubrum), was highly protective against experimental streptococcal infections in mice. The fundamental character of this observation was made clear shortly afterwards by Tréfouël et al. (2) when it was shown that sulphanilamide itself was even more effective than prontosil, and that the latter owed its activity to the fact that it was partially broken down in the body to sulphanilamide. In the hope that compounds more active than the parent substance might exist, many thousands of sulphonamide derivatives have been prepared in the last decade. In one important respect the outcome of this

work has been most clear—namely, that of all the modifications of the parent molecule which have been tried, only by substitution of the hydrogen atoms of the sulphonamide group, $-SO_2NH_2$, can the efficacy of the compound be increased. All other modifications lead to complete or partial loss of activity. In spite of this limitation, sulphonamide derivatives many times more active than the parent compound are in general clinical use.

Sulphapyridine (2-(*p*-aminobenzenesulphonamido)-pyridine). This synthetic compound, often known as M. and B. 693, has the formula :—



Whitby (3) has shown that this pyridine derivative is chemotherapeutically active in relatively small doses against pneumococci of Types I, II, III, V, VII, and VIII in experimental infections of mice. It is especially active against Types I, VII, and VIII, and appears to exert a definite action on the capsule of the pneumococcus. Further, it is as active as sulphanilamide against the hæmolytic streptococcus and meningococcus. It has a low toxicity and does not cause porphyrinuria in the animals tested. It is also efficacious in the treatment of gonorrhœa.

Clinical Applications. Evans and Gaisford (4) have published a series of 200 cases of lobar pneumonia, 100 of which were treated with M. and B. 693. The case mortality was 8%, as compared with 27% in the 100 control cases. Its use in the treatment of lobar pneumonia is now well established. Burford and Blades (5) conclude that the incidence of post-pneumonic empyema has been lowered by sulphonamide therapy. When an empyema occurs it is advisable to discontinue the drug as it masks the temperature, exerts a toxic effect on the patient, and does not affect the pocketed pus. Operation is necessary and pocket formation is liable to occur, with delayed expansion of the lung. Further, in our experience, in some cases although the initial response to treatment appears favourable and the temperature rapidly falls to normal, the pulse and respiration rates remain raised and resolution is delayed. Death may then occur. It has also special value in the treatment of pneumococcal meningitis.

and it is as effective as sulphanilamide in the treatment of streptococcal and meningococcal meningitis. It is occasionally successful in the treatment of staphylococcus aureus infections. The preparation is made up in 0·5 g. tablets, which are crushed and given in suspension in water or milk, or they are swallowed whole. At least 5 pints of fluid a day must be given by mouth to prevent urinary concentration and renal damage from crystallisation. If sufficient fluid is given, it is doubtful whether alkalis are also required. A soluble sodium solution is also put up for intramuscular injection, 3 ml. containing 1 g. This preparation may also be given intravenously, 3 ml. being diluted to 10 ml. with normal saline, the injections being given four-hourly. The intramuscular or intravenous injections are usually only given if the patient cannot tolerate the drug by mouth, but intramuscular injections are best avoided, owing to the risk of local muscle necrosis or of nerve damage.

Pneumonia. Streptococcal and Meningococcal Meningitis. The average adult dose is 4 tablets repeated in four hours, followed by 2 tablets every four hours for two-and-a-half days. Subsequently 1 tablet is given four-hourly for twenty-four hours, then 1 tablet eight-hourly for thirty-six hours, making a total of 23 g. in five days.

For children, gr. 1–1½ (0·06–0·1 g.) per lb. body weight should be given every twenty-four hours. Evans and Gaisford (4) recommend the following dosage :—

Age . .	1–3 months.	6–12 months.	2 years.	3 years.	5 years.
Dosage in Tablets (T.)	¼ T. 4-hourly.	½ T. 4-hourly.	⅔ T. 6-hourly.	1 T. 6-hourly.	1 T. 4-hourly.

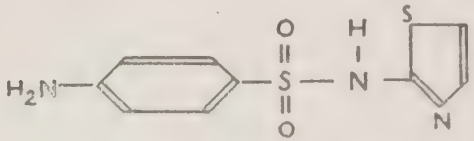
Pneumococcal Meningitis. Hodes, Gimbel and Burnett (6) emphasise the importance of a high initial dose and of subsequent doses sufficient to maintain a concentration of 10 to 15 mg. per 100 ml. in the cerebro-spinal fluid. Very young infants are given 1 to 3 g., and older children and adults 6 to 12 g. by mouth as an initial dose. This is followed by a quarter of the initial dose every six hours until the patient seems entirely well clinically, and several successive cultures of the cerebro-spinal fluid are sterile. This dose should not be reduced until the temperature has been

normal for a week, and half the amount is then given for several days. If the patient cannot swallow, the drug can be administered through a nasal tube. In all cases of pneumococcal meningitis an intravenous injection of a 5% solution of sodium sulphapyridine is also given every six hours, the first dose being on the basis of 0·1 g. per kg. body weight, and subsequent doses of 0·03 g. per kg. body weight until two successive cultures of the cerebro-spinal fluid are sterile. Coleman (7) reports that the case mortality has been lowered by the use of sulphapyridine from 100% to about 35%.

We have found sulphapyridine, given in doses of 1 g. t.i.d. for five days, very satisfactory in the treatment of acute gonorrhœal arthritis, the temperature falling in two days to normal, and the joint swellings and pains disappearing in five days. Sulphapyridine has also been used with beneficial results in the treatment of gonorrhœa, actinomycosis, anthrax, plague, and bacillary dysentery.

Toxic Effects. We have noted severe vomiting in some of our patients who have been treated with this drug. Drug rashes and drug fever are liable to develop if the treatment is continued too long and in some cases after only five days' treatment. Methæmoglobinæmia may occur as a complication, disappearing rapidly when the drug is discontinued. Hæmaturia has also been recorded, especially in warm countries or if insufficient fluid is taken. This may result in anuria and death.

Sulphathiazole (2-(*p*-aminobenzenesulphonamide)-thiazole). The success of sulphapyridine in the treatment of pneumonia encouraged the search for a specific against staphylococci, and sulphathiazole (M. and B. 760) has come into use. It has the formula :—



Sulphathiazole is rapidly absorbed from the intestine and quickly excreted. Reinhold et al. (8) have shown that the blood concentration varies on an average from 3 to 5 mg. per 100 ml. when 4 to 6 g. per day are taken. It is difficult to maintain a higher figure even when larger doses are given.

Clinical Applications. It has been used in streptococcal,

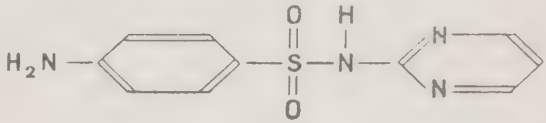
pneumococcal, gonococcal, meningococcal and staphylococcal infections, and workers such as Buttle (9) claim that it is most effective against the last. Long (10) reports from America that sulphathiazole "is about as effective as sulphapyridine in pneumococcic pneumonia in human beings and at least as effective as sulphapyridine, if not more so, in staphylococcal infections in man." Butler (11) treated five cases of severe staphylococcal septicæmia with sulphathiazole, and four died. He concludes that it has little or no effect when the blood culture is positive. It may be of benefit in cases of localised carbuncle or cellulitis or when the septic focus has been removed. Jackman and Martin (12) cured a patient suffering from a carbuncle on the upper lip, with signs suggestive of thrombosis of the cavernous sinus, in which the blood culture was positive. They used sulphathiazole with staphylococcal antitoxin. Melton (13) reports its use in the treatment of six cases of osteomyelitis, one with a positive blood culture; all showed improvement. Gordon (14) treated successfully a severe case of staphylococcal pneumonia, in which previous treatment with sulphapyridine had failed. For gonorrhœa Harrison (15) recommends 5 g. of sulphathiazole daily for four days. Helmholtz (16) reports that sulphathiazole has a definite bacteriostatic effect on streptococcus fæcalis and staphylococcus aureus infections in the urine, whereas sulphanilamide and sulphapyridine have no action on these organisms. Nasal insufflation of sulphathiazole powders has been used by Goldman and Patterson (17) for diphtheria carriers with good results. Banks (18) has used sulphathiazole in the treatment of cerebrospinal fever.

Sulphathiazole is put up in 0·5 g. tablets and the average dose is 4 g., followed by 2 g. four-hourly, up to a total of 30 g. In addition, the 5% sodium solution can be given intravenously in doses of 20 to 40 ml. for an adult, and 10 ml. for a child. A 5% solution of sodium sulphathiazole has been recommended for a nasal spray in the treatment of sinusitis, but it is dangerous owing to its caustic action. The results appear to be as good as those obtained with sulphapyridine, and it is not so toxic.

Toxic Effects. Nausea and vomiting are less common than with sulphapyridine. Drug rashes, conjunctivitis and drug fever are the most common reactions. Hæmaturia, renal failure,

hæmolytic anæmia, and agranulocytosis are occasionally seen. For renal failure large quantities of fluid should be given by mouth, the ureters should be catheterised, and the renal pelvis washed out with warm water at 107° F., every two hours.

Sulphadiazine (2-(*p*-aminobenzenesulphonamido) pyrimidine) has the formula



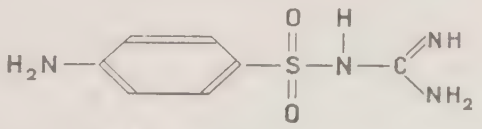
It is absorbed from the intestine more slowly than is sulphanilamide or sulphathiazole, and it is also excreted more slowly. Experimentally it is effective against meningococci, pneumococci, hæmolytic streptococci, staphylococci, Friedländer's bacilli, B. coli and the Cl. welchii in mice.

Clinical Applications. It is used in the treatment of pneumonia, a large initial dose of 0·1 g. per kg. bodyweight being given, followed by 1 g. every four hours by mouth, until the temperature has been normal for seventy-two hours. In children, the initial dose is 0·1 g. per kg. bodyweight, followed by one-quarter of the initial dose every six hours, until the temperature has been normal for forty-eight hours. Dowling et al. (19) treated 115 patients suffering from pneumococcal pneumonia, with a mortality of 11·3%. They gave an initial dose of 4 to 6 g. followed by 1 g. every four hours, until the temperature had been normal for three to four days. Serum was used in addition in some of the cases. Finland et al. (20) had a mortality of 10·7% in 178 cases of pneumococcal pneumonia. They also obtained highly satisfactory results in staphylococcal and streptococcal pneumonia, meningococcal meningitis, erysipelas, acute infections of the urinary tract, and in acute gonorrhœal arthritis. Billings and Wood (21) treated 75 cases of pneumococcal pneumonia with sulphadiazine alone, with only one death. In severe streptococcal and meningococcal infections a concentration of 15 mg. of sulphadiazine per 100 ml. of blood should be maintained. For this purpose the initial dose should be given intravenously, 0·1 g. per kg. bodyweight of sodium sulphadiazine being administered in a 5% solution in sterile distilled water, subsequent doses being 1 g. by mouth every four hours until improvement is

obtained. Sako et al. (22) used the drug in the treatment of influenzal meningitis in children with encouraging results. Five recovered with the drug alone, two others with the drug and antiserum, and two died. Schlesinger and Martin (23) have obtained good results with sulphadiazine and sulphathiazole in the treatment of impetigo, when staphylococci predominated in the lesions. A cure was obtained in 80% of cases in seven days. The daily dosage recommended for six days is 7 g., 6 g., 3 g., 3 g., 3 g., and 2 g. If streptococci predominate, sulphapyridine should be given.

Toxic Effects. These are less common than with the majority of sulphonamide drugs. Nausea and vomiting are rare, but drug fever, rashes, urinary crystal formation, anuria, leucopenia, agranulocytosis, acute hæmolytic anæmia and psychoses may occur. Plenty of fluid should be taken to maintain a daily urine output of 50 oz.

Sulphaguanidine (*p*-amino-N-guanylbenzenesulphonamide) has the formula

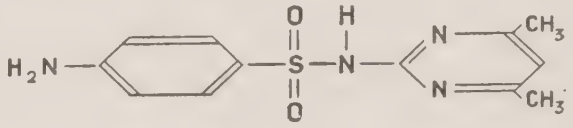


Clinical Applications. As only about one-third of the drug is absorbed from the alimentary tract, it is used especially for the treatment of bacillary dysentery, ulcerative colitis, and gastro-enteritis in children. For an adult 3 to 6 g. may be given by mouth for three days, followed by 2 g. b.i.d. for five days, but in some cases of ulcerative colitis larger doses, such as 4 g. every four hours for forty-eight hours and then 2 g. every four hours, give better results. Anderson and Cruikshank (24) treated 41 cases of acute Flexner dysentery in adults, 2 out of the 28 severe cases in this series dying. Fairley and Boyd (25) report on the use of sulphaguanidine in the treatment of Shiga dysentery. In acute cases the best results were obtained when the treatment was begun within thirty-six hours of the onset. Paulley (26) from his experience of the treatment of bacillary dysentery in the Middle East found that in some instances sulphapyridine had advantages over sulphaguanidine.

Toxic Effects. These are usually slight, but a morbilliform

eruption has been reported by Turell and Leifer (27). Cole (28) observed a severe toxic reaction after administering 110 g. of sulphaguanidine. There was fever, morbilliform eruption, oliguria, hæmaturia and sulphaguanidine crystaluria.

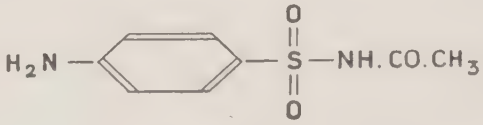
Sulphamethazine (2-(4-aminobenzenesulphonylamino) 4 : 6-dimethylpyrimidine) has the formula



Clinical Applications. Sulphamethazine has been used by Macartney et al. (29) in the treatment of lobar pneumonia, meningococcal meningitis, and gonorrhœa. It appears to be very efficient. An initial dose of 4 g. is given, followed by 2 g. every six hours, or, in more severe cases, 2 g. every four hours. The sodium salt, 1 g. in 3 ml., diluted to 8 ml. with distilled water, can be injected intravenously. It has been used by Jennings and Patterson (30) in the treatment of bronchopneumonia, meningococcal meningitis and lobar pneumonia in children. The dosage varies according to age from an initial dose of 1 to 3 g., with subsequent six-hourly doses of 0·5 to 2 g.

Toxic Effects. Cyanosis does not occur. Nausea and vomiting are infrequent.

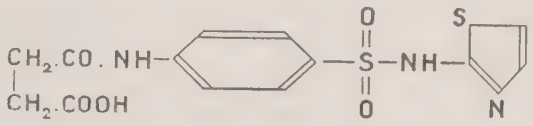
Sulphacetamide (*p*-aminobenzenesulphonacetamide), also known as Albucid or Sulamyl, has the formula



Clinical Applications. Welebir and Barnes (31) found this drug more efficacious in the treatment of *B. coli* infections of the urinary tract than sulphanilamide or the mandelates. The usual dose for an adult is 1 g. t.d.s. for three days, then 0·5 g. t.d.s. for two days, and 0·5 g. b.i.d. for five days. Soluble sodium sulphacetamide, as a 2·5% solution, has also been recommended for application to the eyes after gas poisoning, as a prophylactic against bacterial infection.

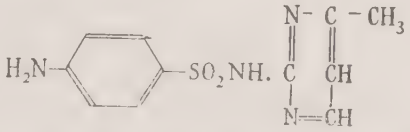
Toxic Effects. Tachycardia, palpitations, or a drug rash may occur in a small proportion of cases.

Succinylsulphathiazole (sulphasuxidine) has the formula



Poth (32) reports the use of this compound, which is poorly absorbed from the alimentary canal, in the pre-operative and post-operative treatment of patients undergoing an operation on the large intestine. It causes a fall in the number of B. coli, Cl. welchii and streptococci in the intestinal contents. The dosage is 0·25 g. per kg. bodyweight daily, divided into six doses and given four-hourly for about five days before the operation, and for a few days subsequently.

Sulphamerazine (2-(*p*-aminobenzenesulphonamido)-4 methyl pyridine) (33) has the following formula :

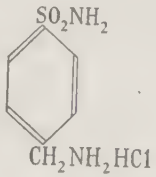


Sulphamerazine has approximately the same antibacterial activity *in vitro* as sulphadiazine and sulphamethazine. It is more quickly absorbed than sulphadiazine and more slowly excreted than sulphamethazine, so that the concentration in the blood rises more quickly and is sustained longer. It is rather more toxic, dose for dose, but in view of the higher blood concentration obtained, this is not significant. Experiments on animals have shown that in high doses it may cause kidney damage, but it is less toxic in this respect than sulphadiazine.

Clinical Applications. According to clinical reports its therapeutic effects are very similar to those of sulphadiazine. Gefter et al. (34) treated 45 cases of meningococcal infections with an average dose of 56 g. over nine and a half days given as follows :— The initial dose was 4 g., followed by 1 g. six-hourly until the temperature had been normal for forty-eight hours, and 0·5 to 1 g. was then given eight-hourly and discontinued on the fifth to eighth day. The mortality rate was 6·7%. A previous series

was treated with sulphadiazine and showed a mortality rate of 12·5%. Lepper, Sweet and Dowling (35) treated 22 cases of meningococcal meningitis with sulphamerazine, and 22 alternate cases with sulphadiazine. There was a slightly quicker defervescence with sulphamerazine, otherwise the results were similar. Hall and Spink (36) treated 116 patients having a variety of infections and found the therapeutic activity and toxicity about the same as sulphadiazine, but lower dosage produced the same result. It was less toxic than sulphanilamide, sulphapyridine or sulphathiazole, but sulphathiazole appeared to be more effective against staphylococcal infections.

Marfanil (△-aminomethyl benzenesulphonamide) has the formula :



This unique sulphonamide is freely soluble in water and is not antagonised by *p*-aminobenzoic acid. German sources recommend the compound for local application to wounds, since the therapeutic effectiveness is not reduced by the presence of pus. It is not suitable for systemic use.

The Mode of Action of the Sulphonamide Drugs

It is the generally accepted opinion that the action of sulphonamides upon susceptible micro-organisms is bacteriostatic rather than bactericidal. By this means the rate of reproduction is reduced and the normal phagocytic defence mechanism is given an opportunity of destroying the invader. The important *rôle* played by leucocytes was emphasised by Fleming (37) who showed that hæmolytic streptococci which were not killed by sulphapyridine alone were rapidly destroyed in the presence of leucocytes. All sulphonamides with the exception of marfanil are antagonised by the presence of small concentrations of *p*-aminobenzoic acid. According to the Fildes-Woods hypothesis (38, 39) this antagonism gives a clue to the mode of action of sulphonamides, namely, that the similarity between the molecules of *p*-amino-

benzoic acid and the sulphonamides is such that they compete for an enzyme system associated with the reproductive phase of the organism. The theory is an elegant one and is supported by the fact that *p*-aminobenzoic acid is recognised as an essential metabolite for many bacteria. However, this does not explain the antagonistic effect displayed by urethane, methionine, peptone, etc., which do not at all resemble the growth factor, nor does it account for the immunity of marfanil. An alternative theory of Henry (40), that the sulphonamides exert their effect by suppressing a respiratory enzyme system, also lacks the complete experimental backing required of a workable hypothesis.

The sulphonamides are amphoteric substances, yet at physiological *p*H the acidic properties predominate. In the blood-stream they behave as salts of weak acids, and as they dissociate, an equilibrium is set up between the ions of the salt and the unionised acid. There is some evidence that the "anion" and not the undissociated acid is responsible for the bacteriostatic effect, and Krebs and Speakman (41) have emphasised the correlation between the potency of the sulphonamides and the strength of the acid which they represent. On the other hand, the different rates of absorption and excretion are factors which do in certain cases exercise an over-riding argument for the use of a particular derivative. Thus, sulphanilamide is suitable for local use; sulphadiazine, because of its slow excretion, establishes a high blood concentration rapidly, whilst the insoluble sulphaguanidine is ideal for infections of the lower alimentary tract.

Sulphonamides, with the exception of those selected for their insolubility, are rapidly absorbed into the blood-stream and are more or less uniformly distributed in the tissues of the body. To a varying extent with different derivatives, the sulphonamides are partially detoxified in the liver by the acetylation of the free amino group. This has clinical importance, since many of these acetyl derivatives have even lower solubility than the parent compound, and tend to crystallise in the renal tubules.

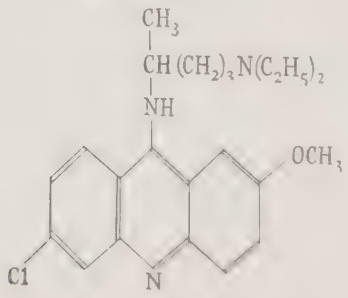
General Considerations. The clinical use of sulphonamides is not without its dangers. When organisms are exposed to concentrations of sulphonamides which are inadequate to inhibit all reproduction, the production of resistant strains is likely. Such strains retain this resistance through many generations, and

although occasionally a more active sulphonamide may be effective, it is now customary to attack such resistance by penicillin therapy.] The risk of producing resistant strains may be avoided clinically by the loading dose which may be administered intravenously or by mouth. [In the case of severe infection likely to endanger life, the intravenous injection is the method of choice, since a high blood concentration is rapidly established.] This high initial blood concentration must be maintained at an effective level by regular oral dosage until all signs of fever have disappeared. As a rule two to three days suffice for most infections, and treatment should usually not be continued for more than seven days. [If desirable, the concentration of sulphonamide in the blood may be followed by chemical assay. It is unwise to give sulphonamide drugs to patients suffering from influenza, and the prophylactic administration of small doses against various types of infection, such as sore throats and colds, is useless. In some cases mental confusion follows a course of sulphonamide treatment.]

One undesirable consequence of the high blood concentrations required for effective therapy is the risk of crystallisation in the renal tubules. Obstruction is less likely to occur if a large fluid intake is maintained and if, furthermore, the *p*H of the urine is kept on the alkaline side. The administration of sodium bicarbonate or sodium citrate is one means of ensuring this.

Synthetic Anti-Malarial Drugs

The first of the synthetic anti-malarial drugs, plasmoquin (pamaquin B.P. Add.), was synthesised by Schulemann, Schoenhoefer and Wingler in 1926. This was followed in 1933 by the synthesis of mepacrine (atebrin) by Mauss and Mietzch. Mepacrine has the following formula :—

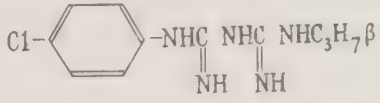


The mode of action of mepacrine is the same as that of quinine ; it affects only the asexual forms of the parasite, so that although the patient can be cured, his blood still remains infectious to mosquitoes. Neither quinine nor mepacrine guarantee against relapse.

Mepacrine produces certain toxic effects such as nausea, vomiting, diarrhœa, headache and anorexia. In about 50% of patients the skin turns yellow. As a rule these effects are not sufficiently serious to warrant discontinuation of therapy.

Mepacrine was used extensively during the war and was issued as a routine measure to troops fighting in areas where malaria is known to be endemic. The dose recommended for prophylactic treatment is 0·1 g. from Monday to Friday, 0·2 g. on Saturday, and none on Sunday, each week while in a malarial infected area. For the treatment of malaria 0·2 g. is given three times a day for three days, and then 0·1 g. for three to four days. This may be followed by pamaquin by 0·01 g. three times a day for four days.

Although mepacrine proved a valuable substitute for quinine in the treatment of malaria, research has been continued in order to find a compound that would be even more effective. Researches were co-ordinated between the Medical Research Council and Imperial Chemical Industries in this country and the Office of Scientific Research and Development in America. This research has led to the synthesis by Curd and Rose (42) of paludrine, which has the following formula :—



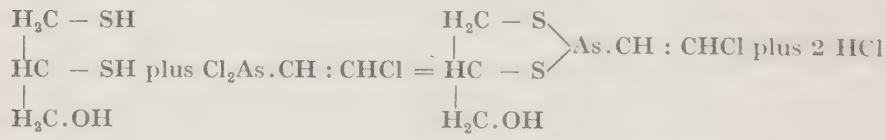
Paludrine is now undergoing an extensive clinical trial in Australia under Hamilton Fairley (43). Preliminary investigations show that it is an effective clinical suppressive in both malignant and benign tertian malaria. It appears also to be a causal prophylactic in malignant tertian malaria but is not so effective in this respect as in benign tertian malaria. Paludrine is also effective as a curative agent in both malignant and benign tertian malaria and in quartan malaria. Its mode of action appears to be somewhat different from that of quinine and mepacrine, as under certain circumstances it appears to be able

to prevent the gametocytes from maturing when taken up by mosquitoes. The toxicity of paludrine has been found to be negligible in the doses so far used. Adams et al. (44) treated 147 cases of acute benign tertian with paludrine in doses ranging from 10 mg. to 700 mg. twelve-hourly for fourteen to twenty-eight days. Clinical cure was obtained in all cases. They also treated 22 cases of malignant tertian malaria with doses of 50 mg. to 600 mg. twelve-hourly for fourteen days with control of the attacks.

British Anti-Lewisite

An account of the work which led to the discovery of this substance has been published by Peters, Stocken and Thompson (45). It was suggested by the previous work of Walker (46), Voegtlin, Dyer and Leonard (47) and Cohen, King and Strangeways (48) that the toxicity of certain arsenical drugs is due to their attack on — SH groups. Peters (49) had shown that certain vesicant substances, such as dichlordiethyl sulphone and iodoacetic acid, had a toxic effect on the pyruvate component of the enzyme system oxidising lactate in the brain, producing effects similar to those of vitamin B_1 deficiency. By an association of these ideas, Peters, Stocken and Thompson were led to explore the possibilities of certain dithiols, in the hope that these would form with lewisite and other arsenicals, compounds more stable than those formed by the thiols in the body.

British anti-lewisite, discovered as a result of this work, is 2, 3-dimercapto propanol and its reaction with lewisite is :



It was found that this substance not only prevents the toxicity of lewisite, but can also reverse it. Experiments on volunteers at Porton showed that vesication could be prevented one hour after contamination by the application of BAL, and erythema and œdema would begin to diminish in twenty-four hours. A marked increase in the urinary excretion of arsenic was found. The substance has been used with good effect in factories manu-

facturing lewisite. It also has possibilities as an antidote to poisoning by other arsenical compounds such as mapharside, but its use in clinical medicine for this purpose is still in the experimental stage.

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CHAPTER II

PENICILLIN AND SOME OTHER ANTIBIOTIC SUBSTANCES

Penicillin

The last few decades have witnessed the introduction of an increasing number of chemical compounds which, whilst showing toxicity of a high order against pathogenic organisms, exercise little or no harmful effect upon their human hosts. The period has been one of intense activity in the field of organic chemistry and by far the majority of compounds tested have been of synthetic origin. It is remarkable in these circumstances that penicillin, in many respects the most important therapeutic agent at the present time, should come from the laboratory of a bacteriologist.

Penicillin was discovered by Fleming in 1929 (1). It is an extracellular product of the metabolism of the mould *Penicillium notatum* and represents one of the first of a series of antibacterial compounds obtained from moulds and bacteria. Many of these compounds display potency of a very high order when tested *in vitro*; relatively few are sufficiently free of toxic effects on the host to permit their therapeutic application.

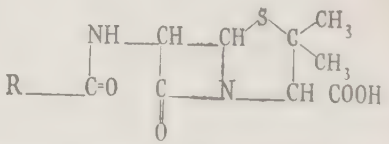
Production of Penicillin. The modern methods of large-scale penicillin production differ from the original small-scale extraction of Florey and co-workers (2, 3) in technical details only. The principles involved are similar, and no mould superior to the original strain used by Fleming has been found until very recently. *Penicillium notatum* grows well on a simple medium of glucose and a few inorganic salts. The surface culture of *penicillium notatum* No. 1249 B 21 is extensively used in England, whilst the deep culture technique, using *penicillium notatum* No. 832, has been developed on a vast scale in the United States. Early in 1946, Rapier and Alexander (4) announced the development of a new mutant of *penicillium notatum* by irradiation with ultra-violet light. This new mould variant gives a high yield of penicillin with at least half of it in the penicillin III form (see p. 18).

The inoculation of the medium must be carried out under sterile conditions and the incubation takes place at 20° to 26° C. As the mould grows, the reaction of the medium falls initially from neutrality to *p*H 4 to 5, and subsequently rises to *p*H 8 at the end of seven days. Penicillin production is associated with spore formation and with the simultaneous appearance of a pigment, chrysogenin. Frequent assays carried out by the "Oxford cup method" serve to indicate the peak of penicillin production and the active principle is extracted from the filtered medium by organic solvents. The material so obtained is crude and further purification by chromatographic absorption and elution is essential to obtain a specimen suitable for clinical application.

Chemical Constitution. The establishment of the constitution of the penicillin molecule has been a major war-time task in which many investigators, at present unnamed, have collaborated (5). It is now clear that the mould product is not a separate entity, but consists of a number of closely related chemical compounds, all of which may be represented by the general formula $C_9H_{11}O_4SN_2R$. Molecular weight determinations, which show that this empirical formula also represents the molecular formula, in each case reveal the relative simplicity of the basic penicillin structure. The compounds in the following table have been obtained in the pure state :

English Name	American Equivalent	R
Penicillin I	F Penicillin	$-CH_2.CH=CH.CH_2.CH_3$ Δ_2-pentenyl
Dehydro-penicillin I		$-CH_2.CH_2.CH_2.CH_2.CH_3$ n-amyl
Penicillin II	G Penicillin	$-CH_2.C_6H_5$ benzyl
Penicillin III	X Penicillin	$-CH_2.C_6H_4.OH$ p-hydroxybenzyl
Penicillin IV	K Penicillin	$-CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_3$ n-heptyl

Accumulating evidence points increasingly in favour of the following formula for the common constituent of the penicillin molecule :—



The penicillins when pure are white crystalline solids, soluble in water (5 mg./ml.), alcohol, acetone, ethyl acetate, etc. Solubility

in ether, benzene and chloroform is low. In aqueous solution they behave as strong mono-basic acids and readily give salts with the alkali metals and earths. The sodium and calcium salts are used clinically. In solution, penicillin is sensitive to extremes of *p*H (stable *p*H 5–7) and to excessive rise of temperature. Oxidising agents and traces of certain heavy metals accelerate the decomposition. Even in the dry state it is recommended that penicillin should be stored below 10° C.

Penicillin in solution is rapidly decomposed by *Penicillinase*, an active principle produced by many bacterial cultures. This substance, as its name implies, behaves as an enzyme on the substrate penicillin; it exhibits optimum activity between *p*H 5 and 7 at 37° C., but is completely destroyed at 50° C. Although unequivocal evidence is lacking, the possibility that the inherent and acquired resistance of organisms to penicillin is related to their ability to produce penicillinase cannot be excluded.

Assay of Penicillin. The microbiological assay of penicillin by the "Oxford cup method" can be applied to media of both industrial and biological origin. The value of such assays to the producers and consumers of penicillin is obvious. According to the original method described by Abraham et al. (3), 100 ml. of nutrient agar are inoculated with 0·1 ml. of a broth culture of staphylococcus aureus H. and are plated out in a series of sterile Petri dishes. Small cylinders of resistance glass (8 mm. diameter × 12 mm. length, wall thickness 1 mm.) are sterilised by flaming, and sealed on to the agar plate by resting the still warm base of the cylinder against the gel surface. Six cylinders of this type are uniformly dispersed on each Petri dish and the cups so formed are filled with six dilutions of the experimental fluid. In an analogous manner a similar plate is prepared using six dilutions of a standard penicillin solution. The Petri dishes are incubated at 37° C. for twenty-four hours.

The diffusion of the antibiotic principle from the cups into the medium inhibits the growth of the cocci over an area of the agar surface which is related to the concentration of penicillin in the sample. The diameters of the inhibition zones are measured and a standard curve prepared. By reference to this standard curve, the potency of the experimental fluid may be assayed. An accuracy of ± 10% is claimed for the method.

Until the establishment of a standard of pure penicillin, the activity of most preparations is expressed in terms of the Oxford unit, which in turn is defined in terms of a standard penicillin preparation maintained by the Oxford workers.

Antibiotic Activity. Whilst the clinical experience of penicillin is closely in accord with the results of *in vitro* experiments, penicillin is not the universal panacea. In general it is most active against Gram-positive organisms, and in particular against cocci rather than bacilli. Promising results have also been obtained with several pathogenic spirochætes, but with few exceptions virus, mould, trypanosome and helminthic infections do not respond to penicillin treatment, nor are the organisms destroyed *in vitro*. The following table classifies the commoner organisms according to sensitivity to penicillin :—

Sensitive

Gram-positive cocci
Staph. aureus (most strains)
Staph. albus
Str. pyogenes
Str. viridans (most strains)
Str. pneumoniæ

Neisseriæ
N. gonorrhœæ
N. meningitidis

Clostridia
Cl. welchii
Cl. septicum
Cl. œdematiens
Cl. tetani (see p. 34)

Viruses
Psittacosis
Ornithosis
Lymphogranuloma inguinale

Treponemes
Trep. pallidum
Trep. recurrentis
Trep. vincenti

Miscellaneous
B. anthracis
C. diphtheriæ
Actinomyces bovis (some strains)
Leptospira icterohæmorrhagiæ

Slightly sensitive
S. gærtneri
Vibrio El Tor

Insensitive
Ps. pyocyanea
Proteus
Bact. coli
Bact. friedländeri
Bact. typhosum
Bact. paratyphosum
Bact. sonnei
Bact. shigæ
Bact. flexneri
H. pertussis
H. influenzæ
Past. pestis
Brucella abortus
Brucella melitensis
Str. fæcalis
Str. viridans (some strains)
Staph. aureus (some strains)
M. tuberculosis
Vibrio choleræ

The mode of action of penicillin is unknown, but it is certain that the major effect is on the organism rather than on the host. The character of the attack on sensitive organisms, at least *in vitro*, varies both with the concentration of penicillin and the phase of growth of the bacterial colony. Thus, when bacteria are grown on a rich medium under conditions of temperature most favourable to their growth, the bactericidal action of penicillin is at a maximum.

According to Gardner (6), small amounts of penicillin, one-tenth of the bacteriostatic dose, produce extensive morphological changes in bacteria. Cocci swell to a very great size, whilst bacilli elongate without division. This manifest bacteriostatic effect is supported by the findings of Chain and Duthie (7), who have studied the effect of adding penicillin to cultures of staphylococci at various stages of growth. Added at the early lag phase, penicillin has little effect on population numbers, but the medium becomes turbid owing to the production of enlarged forms which are apparently unable to divide. Added during the logarithmic phase, penicillin produces no immediate effect, but after ten minutes there is a rapid lysis and killing of newly-formed cells. It is assumed that this ten minutes is the time taken for the cells to go through one division. Chain et al. suggest that penicillin interferes with a metabolic function involved in the early stages of bacterial development. Penicillin added at the resting phase of growth is without effect on number or form.

A factor of paramount importance to the clinical application of penicillin is that the antibiotic activity is in no way diminished by the presence of serum, pus or tissue debris. In this respect penicillin differs significantly from the sulphonamides. Not only is there a complete absence of competitive antagonism in the presence of *p*-aminobenzoic acid, but many workers such as Bigger (8), and Kirby (9), maintain that penicillin in the presence of *p*-aminobenzoic acid or sulphonamides shows synergistic action against Gram-positive cocci.

Resistant strains of organisms otherwise susceptible to penicillin result from inadequate penicillin therapy. Although such resistance occasionally behaves as a hereditary factor as shown by Schmidt and Sesler (10), it is not normally associated with increasing virulence. On the contrary, Gram-positive cocci

rendered penicillin-resistant *in vitro* are not only sensitive to penicillin *in vivo*, but are more so than the parent strain. It is indeed fortunate that organisms rendered resistant to sulphonamides *in vivo* succumb to penicillin therapy. The reverse is also generally true.

Administration. The development of penicillin-resistant strains as a result of inadequate therapy emphasises the necessity of maintaining a sufficiently high penicillin level in the blood. A figure of from 0·02 to 0·20 units per ml. of serum is required, and although this can easily be achieved by intravenous administration, the rapid elimination of penicillin in the urine prevents this figure from being maintained except by very frequent injections. In practice, using watery solutions and average doses, eight intramuscular injections every twenty-four hours are needed to ensure bacteriostasis and at times the method of continuous drip is to be preferred. The penicillin in such cases is made up in isotonic saline, in 5% dextrose solution, or it may be administered simultaneously with a blood transfusion. In this way as much as 200,000 units may be given in twenty-four hours to augment the high concentration of an initial intravenous injection. It is impossible to give hard and fast rules for the optimum conditions of administration which cover all cases. Preliminary reports of the Penicillin Clinical Trials Committee (11) recommend the administration of 60,000 units injected intramuscularly every three hours, the duration of treatment varying according to the infection from a minimum of twenty-four hours in cases of uncomplicated gonorrhœa to twenty-eight days in severe bacterial endocarditis.

It has been known for some time that in cases of renal insufficiency high concentrations of penicillin in the blood can be maintained over long periods, and this has led to the practice of inducing temporary excretory blockade by raising the renal threshold for all substances excreted by the tubules. Beyer et al. (12) have achieved this blockade by injection of sodium aminohippurate or phenol red.

Injected intramuscularly, a maximum concentration is obtained in the blood within fifteen minutes, and with adequate doses, is maintained for three to four hours. The intravenous route has therefore little to recommend it, and frequently has to be dis-

continued after forty-eight hours owing to thrombo-phlebitis. The subcutaneous route, whilst ensuring slow excretion, is more erratic, is not without painful effects, and is seldom used. Penicillin, when administered orally, is largely destroyed by the acidity of the gastric juice. By enclosing penicillin in gelatin capsules coated to resist peptic attack, some may pass through the pyloric sphincter, but, even so, any surviving penicillin is exposed to the destructive effect of penicillinase formed by the normal bacterial flora of the intestinal tract. For the latter reason rectal administration is unsuitable. Despite this, if penicillin is given in large doses, sufficient is absorbed into the blood to deal with the most penicillin-sensitive organisms, the gonococcus and the pneumococcus, and in some cases, the staphylococcus. Finland, Meads and Ory (13) showed that a dose of 90,000 units of penicillin given in water by mouth half an hour before breakfast regularly gave serum levels comparable with those obtained from 15,000 or 20,000 units given intramuscularly. The serum levels are higher and more sustained in cases of achlorhydria. Effective serum levels were obtained with oral doses of 90,000 units every two hours. Bunn et al. (14) treated patients suffering from pneumonia with oral penicillin, administered either suspended in corn or cotton seed oil in a gelatin capsule, or dissolved in 30 ml. of iced tap water. There was one death in 45 cases. The average dose was 750,000 units on the first day, and 400,000 to 600,000 daily, subsequently, for seven or more days. Ross et al. (15) used double capsules for the oral administration of penicillin. 100,000 units of powder were placed in a plain gelatin capsule, which was sealed and inserted in a larger gelatin capsule. The latter was hardened by formaldehyde and 95% alcohol. Two aluminium hydroxide tablets were given half an hour before swallowing the capsule. The capsules were swallowed every three hours. Ten children with gonorrhœa, two with pneumonia, and two with cellulitis were rapidly cured.

Penicillin administered by any of the above routes gains access to most organs of the thoracic and peritoneal cavities, the kidneys, liver, intestines, pancreas, heart and lungs. In bile the concentration is sufficiently high to permit direct penicillin therapy for biliary infections, providing the organism is sensitive. The absence of substantial concentrations in bone marrow, brain

and cerebrospinal fluid, however, calls for special administration. The continuous drip technique lends itself well to the intramedullary route, whilst intrathecal injection gains access to both the neural canal and the cerebral meninges. There is little doubt that penicillin is transmitted through the placenta, and that the concentration in the fœtal circulation approaches that of the maternal circulation.

For localised infections such as wounds, empyemata, etc., penicillin can be applied locally, and for this purpose is available in three forms, as a solution, a powder or a cream. As in the case of general infection, the important point is to ensure a sufficiently high concentration to inhibit the growth of the organisms. For deep wounds, sinuses, cavities, etc., the best method is to aspirate fluid or pus and replace it with penicillin solution.

Apart from a slight risk of thrombosis, there are no contra-indications to the use of penicillin. The nausea and fevers are mild and the allergic skin reactions, urticaria and dermatitis, may well be due to impurities in the penicillin preparation. If infection is due to a sensitive organism, and if adequate supplies are available, the administration of penicillin is at least a wise precaution.

Clinical Applications

In the space available it is impossible to give a full account of the numerous applications of penicillin. Despite the limited supply of material, a formidable literature has already been devoted to applications, successful and otherwise, chiefly against coccal infections in their manifold forms. Results against the anærobes of the Clostridium group as well as against the spirochæte of syphilis are also promising. New additions to the list of susceptible organisms, and improvements in clinical technique are almost certain in the future.

Preparations of Penicillin. *Powder* (sodium and calcium salts). This is put up in rubber-capped vials containing 100,000, 200,000, 500,000 and 1,000,000 units. A mega unit is 1,000,000 units. This powder is used for preparing solutions for injection and inhalation. *Tablets*, each containing 1,000, 8,500 or 10,000 units. *Oily injection.* A sterile suspension of the calcium salt in white

beeswax and arachnis oil is supplied in rubber-capped vials. The dose is m. 15 to 60, which contains 125,000 to 500,000 units, *i.e.*, 1 ml. contains 125,000 units. *Lozenge or pastille*, each containing 500 units of penicillin in a gelatin or acacia base. *Ointments and creams.* Penicillin may be incorporated in a cream of 30% Lanette wax S.X. in water, containing 500 units per gramme. Lanette wax S.X. is a sulphated fatty alcohol, used as an emulsifier. Creams generally contain more water than do ointments, and are therefore less stable. The unsterilised ointments usually contain phenoxetol to prevent the growth of organisms producing penicillinase, especially the Ps. pyocyanea. Ointments usually contain soft paraffin and/or wool fat. The B.P. preparations are a cream, a sterilised cream, an ointment for the eye containing 1,000 units per gramme, and an ointment. *Powder for local use.* Penicillin is mixed with sterile sulphathiazole or sulphanilamide to give a concentration of 5,000 units per gramme. *Eye-drops.* These contain 500 to 2,000 units per ml. *Eye "Tabloids,"* each containing 250 units.

Penicillin is rapidly destroyed by heat. Its stability varies according to the preparation. Solutions for injection should be used within forty-eight hours of preparation and should be kept at a temperature not exceeding 40° F. (4° C.) during this period. Creams should be stored in a refrigerator at 50° F. (10° C.), but when taken out they remain effective for one week if kept in a cool place. Penicillin powder, lozenges and ointments, can be stored for a year in a cool, dry place, such as a dry cellar, if the temperature does not exceed 60° F. Thus the doctor who has no refrigerator can keep a supply of penicillin powder in rubber-capped vials, also ointments and lozenges.

The calcium salt is recommended for use in oily preparations, either as an intramuscular or intratracheal injection. As a powder, mixed with a sulphonamide drug, it is less hygroscopic and so more stable than the sodium salt. The calcium salt is also used, dissolved in water and vaporised, for inhalation, as it has less odour and less tendency to produce cough than sodium penicillin. The sodium salt is generally used for intramuscular and intrathecal injection. Some authorities consider that the calcium salt is more irritant than the sodium one and should not be used in watery solution for injection.

Intramuscular Injection. A dose of 15,000–60,000 units of penicillin is given every three hours, or a single daily dose of 100,000 to 150,000 units. The dose is prepared by adding 5 ml. of pyrogen-free sterile normal saline or distilled water to the vial which contains 100,000 units of penicillin powder. This gives a dose of penicillin of 20,000 units per ml. Pyrogen-free distilled water is freshly distilled water which has been immediately autoclaved in a sealed vessel. The sterile water is conveniently put up in rubber-capped vaccine bottles containing 10 or 50 ml., or in ampoules containing 10 or 20 ml. The method is as follows:—

The syringe and needle must be sterilised, and this may be done by boiling them in tap water. They may be left in sterile distilled water until ready for use. They must never be placed in spirit or any other antiseptic solution. The syringe is then filled with the saline or distilled water and the requisite amount (5 ml.) injected into the vial which has a rubber cap protected by a metal cover. This cover is removed and the rubber cap cleaned with ether. The vial is shaken to dissolve the penicillin and the fluid is sucked up again into the syringe and is ready for injection.

The skin is prepared with soap and water, or saline, or ether, or Cetavlon. The latter is 1% cetyl-trimethyl ammonium bromide (I.C.I.).

One to 3 ml. of the prepared penicillin solution is drawn into a syringe and an Arnold No. 6 serum needle, or a 2 inch × 23 gauge needle, or a stainless steel needle size 6 or 7 (Firth), attached. The stainless steel is less liable to break. For the oil-wax suspension a 26 gauge needle may be used. The needle should be inserted quickly, not quite up to the hilt, in the upper and outer quadrant of the buttock in order to avoid the sciatic nerve. This is of great importance. It must not be inserted obliquely, as penicillin is poorly absorbed from the subcutaneous tissues. The second injection should be given in the opposite buttock. Alternative sites, especially in males in whom the buttocks may be thin, are the lateral side of the thighs at the junction of the middle and upper third, the deltoid and the triceps muscles. The solution should be cold when injected. Intramuscular injections are sometimes painful. This reaction varies with the skill of the operator, from patient to patient, from batch to batch of penicillin, and with the quantity of fluid injected. If there is pain, penicillin

may be given with procaine (Novocain), 0·5 to 1 ml. of 2% procaine being drawn up into the syringe containing the dose of penicillin. The injection is made slowly and the procaine precedes the penicillin. Sodium amytal, gr. 3 at night, is helpful in minimising the disturbance from the injections.

Injection by continuous intramuscular drip is now seldom used, and will not be described. It has the advantage of requiring less attention at night.

Powder for Local Treatment. Dry penicillin powder made up with sterile sulphathiazole is used for wounds and abrasions. The powder is blown on to the surface with an insufflator to form a thick hoar frost. The application should be repeated every twenty-four hours, but must not be continued for longer than five days, owing to the risk of the development of skin reactions due to sensitivity to the sulphonamide. This method is of most use in relatively dry surfaces, as blood or exudate tend to wash away the powder.

Ointments and Creams. These are used for local application in certain skin or eye infections (see p. 34).

Lozenges or Pastilles. These are useful in treating infections of the mouth and throat. One pastille should be sucked every hour for 8 doses (see p. 35).

Eye Drops. In certain eye infections drops containing 500–2,000 units per ml. every one or two hours are beneficial (see p. 35).

Inhalation. Penicillin may be given by inhalation, in the treatment of bronchitis and bronchiectasis (see p. 30), by means of an inhaler, such as the Collison. Twenty thousand to 100,000 units in 2 ml. of distilled water are put into the bulb for each inhalation, and the " mist " inhaled every two hours daily through the oronasal mask furnished with an expiratory valve. The vaporisation is effected by oxygen and takes about fifteen minutes. The rubber tubing of the apparatus should not be a synthetic preparation, which inactivates the penicillin.

Solutions. These can be made up with distilled water or saline, at 1,000 units per ml. for instilling twelve-hourly through fine rubber tubes into wounds which have been closed, for injecting into abscess cavities after aspiration, and as a spray for skin and mucous membrane infections. Solutions of a strength of 1,000 to 2,000 units per ml. are suitable for injecting into the cerebro-

spinal, pleural, pericardial and joint cavities, and 10,000 units per ml. for intratracheal injection.

Septicæmia. Successful treatment of septicæmia, whether of staphylococcal or streptococcal origin, necessitates not only the complete sterilisation of the blood, but also the elimination of primary and secondary foci of infection. It is recommended that an initial massive dose of 100,000 units be given intramuscularly, followed by intravenous injection of 200,000 units daily for from three to five days. If thrombophlebitis occurs the injections must be given intramuscularly, 30,000 units every three hours. Adjuvant treatment by the oral administration of sulphonamides has reduced the mortality from 90% before the advent of chemotherapy to 35%. The β-hæmolytic streptococci group vary in sensitivity both to penicillin and sulphonamides. The subgroup A of Lancefield responds to penicillin therapy, but the drug is less effective with other subgroups. This is especially true of subgroup D which includes several highly resistant strains.

Subacute Bacterial Endocarditis. Less success has been achieved in the treatment of this disease due in many cases to infection by organisms of the viridans group. That sulphonamide therapy has not been successful is apparent, since post-mortem examination has revealed the continued presence of live organisms in the cardiac lesions. Following the administration of massive doses of penicillin, sterile blood cultures have been reported from infections of streptococcus salivarius and streptococcus fæcalis. This in itself cannot be regarded as satisfactory and patients should be free from all clinical symptoms for a substantial time after the completion of treatment. Loewe et al. (16) recommend the simultaneous injection of penicillin and heparin, the latter being used to break up the fibrin barriers which normally protect the organisms from therapeutic attack. Rather more encouraging data are given in the report of Christie (17, 18) on the treatment of 147 patients in fourteen different centres appointed by the Penicillin Clinical Trials Committee. The patients were divided into different groups in order to ascertain what system of dosage was most likely to achieve permanent results. It was found that the duration of treatment was of greater importance than the total amount of penicillin given, and that patients receiving doses of 500,000 units per day for twenty-eight days were more likely to

do well than those receiving a larger dose for a shorter time. Eighty-one of the patients were apparently cured and 50 have died. The penicillin was given either by intramuscular injections every three hours, or by continuous intramuscular drip. No difference in the therapeutic results was noted in the two methods. Many patients who had relapsed after previous treatment with penicillin were given further courses. Of 26 patients who had previously had short courses (five to ten days), 15 recovered after a further course of twenty to twenty-eight days. Of 26 patients who relapsed after long courses (twenty to twenty-eight days) only 8 recovered after a further course. Better results have been obtained in America by Bloomfield and Halpern (19) by giving 50,000 units intramuscularly every six hours for two months.

Meningitis. Successful treatment of meningitis by the administration of penicillin and sulphonamides has been reported by many workers including Rosenberg and Arling (20) and Daniels et al. (21). Staphylococcal infections of the meninges are usually secondary to mastoid and sinus foci. Immediate surgical drainage of such centres, followed by local treatment with penicillin solution, is of considerable value in reducing the chance of reinfection. Smith, Duthie and Cairns (22) advise the treatment of uncomplicated pneumococcal meningitis as follows: After finding pneumococci in the cerebro-spinal fluid 8,000 to 16,000 units of penicillin are injected intrathecally, with smaller doses for infants. The penicillin is in pyrogen-free saline solution, 2,000 units per ml. Fifteen thousand units of penicillin are injected intramuscularly every three hours to control any primary infection or septicæmia. Sulphadiazine is also given by mouth or nasal tube, first 4 g., and then 2 g. every four hours, an adequate fluid intake being ensured to prevent renal block. After twelve hours a second intrathecal injection of penicillin is given. If the fluid is now thicker a third puncture should be made twelve hours later to detect any incipient ventricular block, which might require intraventricular injections of penicillin. Daily injections of penicillin into the theca must be continued for five days, the intramuscular injections are discontinued after five days, and the sulphadiazine reduced to 1 g. four-hourly after five days and maintained at this dose for a week.

If the cerebro-spinal pathway becomes blocked by pus, in sites such as the aqueduct, penicillin should be injected into the lateral ventricles through parietal burr-holes. After draining the pus the penicillin can be injected through an in-dwelling catheter. In all cases in which intrathecal injections are required non-irritating preparations of penicillin should be used, and great care taken in the aseptic technique. Of 34 fully treated cases, 5 died and 27 recovered completely. In meningococcal and streptococcal meningitis similar amounts of penicillin are injected intrathecally every twenty-four hours until the cerebro-spinal fluid is free from organisms, and intramuscular injections are given every three hours of 20,000 units for two or three days.

Pneumonia. In the treatment of pneumonia there is little to choose between penicillin and the sulphonamides, except in cases of sulphonamide resistance especially those due to staphylococci, or where the lower toxicity of penicillin is advantageous. The usual dose for an adult is 20,000 to 30,000 units every three hours for seven days. For a child, 1,000 units for each year of age should be injected every three hours. Primary atypical pneumonia does not respond to penicillin.

Empyema. In the treatment of empyemata due to pneumococci, streptococci or staphylococci, but not in cases of b. coli infections, also in infected hæmothorax following injury, intra-pleural instillation is the method of choice. Penicillin solution containing 1,000 units per ml. should be injected into the pleural space following aspiration of pleural fluid. The amount of penicillin injected depends on the size of the empyema cavity. Cavities holding 10 to 20 oz. of fluid should receive 60,000 units of penicillin, total empyemata 120,000 units, and 30,000 units for small pockets. Three to four aspirations and instillations generally suffice. Thoracotomy is usually required to evacuate blood clot, fibrin and pus. Local penicillin treatment after rib-resection is often unsatisfactory and penicillin is then best given by eight-hourly intramuscular injections of 100,000 units.

Bronchiectasis. The cavities are emptied as far as possible by postural drainage, followed by the intratracheal injection of penicillin. The skin and subcutaneous tissues are anæsthetised in the neck, between the cricoid cartilage and the first ring of the trachea, with 2 ml. of 2% Novocain. Two ml. of 2% Decicain

are then injected rapidly through a No. 14 needle into the trachea and the patient told to avoid coughing. The syringe is detached from the needle, the patient placed in the optimum position for fluid to run down the trachea to the affected part of the lung, and then 8 ml. of penicillin solution containing 10,000 units per ml. are rapidly injected through the needle which has been left *in situ* in the trachea. This treatment, repeated daily for seven days, has resulted in considerable clinical improvement. Alternatively the penicillin can be given every two hours by inhalation (see p. 27).

Chronic Bronchitis. In some cases the amount of sputum can be very considerably reduced and the breathing made easier, by the inhalation of penicillin, as described above (see p. 27).

Infections of the Skeletal System. Osteomyelitis and cocci infections following compound fractures respond well to both penicillin and sulphonamide therapy. It is on record that cases of chronic osteomyelitis of some years' duration have been cleared in a relatively short period by penicillin treatment. Sterile blood cultures may be obtained following intravenous, supported by intramuscular injection, but the intramedullary route is obviously recommended for resistant strains. Grace and Bryson (23) treated 7 cases of chronic osteomyelitis, with 1 failure. The penicillin was given intramuscularly in doses of 30,000 units every three hours for ten to twenty-one days, and simultaneously 4,000 to 20,000 units of penicillin per ml. were applied locally into the sinus tract. In open wounds, aspiration of exudate and local administration of penicillin and sulphonamide powder accelerate the recovery. Kirby and Hepp (24) have emphasised the risk of secondary meningococcal infection from osteomyelitis of the facial bones, and recommend penicillin rather than sulphonamides in order to reduce the risk of reinfection. Penicillin treatment of osteomyelitis should be followed by surgical removal of infected or necrotic bone. Infections by streptococcal and staphylococcal organisms located in bone, joints and synovial membranes respond well to prolonged penicillin treatment. In contrast, chronic rheumatoid arthritis, even when associated with recognised coccal strains known to be penicillin-sensitive, fails to respond to what must be regarded as massive doses of the drug. Boland, Headley and Hench (25), who have given careful

attention to classical cases of arthritis, report complete failure.

Florey and Florey (26) recommend the daily application of penicillin in the wound following mastoidectomy.

Gonorrhœa in the Male. The efficacy of penicillin treatment in gonorrhœa has been established beyond doubt. Even when examined over a period of several weeks, patients show no recurrence of symptoms. Welch et al. (27) report the superiority of penicillin X in the treatment of gonorrhœa by single intramuscular injections of 25,000 units, but many other workers suggest that 100,000 units of penicillin or even more are essential to ensure a permanent cure in all cases. Most strains of the organism are sensitive to sulphathiazole treatment, but are prone to develop sulphonamide resistance. Fortunately the sulphonamide-resistant strains which are encountered show no penicillin resistance. Five injections of 20,000 units every two hours are usually sufficient to effect a cure. The difficulty of maintaining a sufficiently high blood concentration by one injection in out-patients who cannot attend for the five injections, has been overcome by Romansky and Rittman (28) who recommend a beeswax-peanut oil mixture as the medium for injection. By this means they cured 11 out of 12 patients suffering from gonococcal urethritis with a single injection of penicillin of 40,000 to 66,000 units. Romansky et al. (29) obtained 100% of cures in 75 cases of gonorrhœa in males, by a single oil injection of 175,000 units of penicillin. Whatever treatment is given the patient should be re-examined for infection after two and four months.

Gonorrhœa in the Female. This responds equally well to penicillin therapy, symptoms disappearing in the majority of cases following the administration of 100,000 units.

Syphilitic Infections. In primary and secondary syphilis penicillin therapy has proved equally satisfactory. The results obtained by many workers compare well with those using massive arsenical therapy, without exposing the patient to the toxic effects and idiosyncrasies of the latter drugs. Whether penicillin is given intramuscularly or intravenously, a continuously high concentration in the blood must be maintained. Mahoney et al. (30) in an early communication, showed the disappearance of the spirochætes and clearing of serological reactions following the administration of 25,000 units every four hours for eight days.

Moore et al. (31) and Norcross (32) confirm this finding and have reported apparent recovery from primary and secondary syphilis following treatment with much lower quantities of penicillin. Leifer (33) from his experience in treating 96 cases, concluded that early syphilis can be cured by intramuscular injections of 20,000 units every three hours for seven and a half days, giving a total of 1·2 million units. Lourie et al. (34) have reported favourably on a method for the ambulatory treatment of early syphilis. Three intramuscular injections of 600,000 units were given at hourly intervals on five consecutive days. The penicillin was dissolved in 5 ml. water. The treatment of cardio-vascular syphilis with penicillin has proved unsatisfactory in the hands of Dolkart and Schwemlein (35), the injections having to be abandoned owing to cardiac pain. The simultaneous administration of arsenicals and penicillin has been claimed to have a synergistic effect and to be superior to straightforward penicillin therapy. The Committee on Medical Research and the United States Public Health Service (36) reported in 1946 on the treatment of early syphilis with penicillin. They found that the percentage failure at the end of eleven months after treatment varied from 15% when 2,400,000 units of penicillin had been given, to 62% with 600,000 units. Patients treated during the first week of disease did twice as well as those treated after two months or later. Simultaneous treatment with arsenic or bismuth increased the number of patients cured when total amounts of penicillin were given varying between 300,000 to 1·2 million units.

Gas Gangrene. The administration of penicillin to subjects with wounds infected by organisms producing gas gangrene has been reported from several sources. No clear indication of the effectiveness of penicillin alone is possible, since the administration has always been accompanied by injections of antitoxin, local administration of sulphonamides, and, where necessary, by surgical operation. Although Cutler (37) has reported 4 cases of gas gangrene infections following compound fractures which failed to respond to intravenous injection of penicillin, the consensus of opinion in this matter is that local administration of penicillin is at least as effective as any preceding method, and that intravenous administration of the drug prior to operation is an effective means of localising the area of attack.

Tetanus. *In vitro*, Cl. tetani is sensitive to penicillin. Altemeier (38) did not find that penicillin had any beneficial influence on the course of established clinical tetanus. This is to be expected, as penicillin has no known effect on the tetanus toxin.

Agranulocytosis. Boland et al. (39) have shown that penicillin is of value in treating agranulocytosis due to various causes, as death generally results from secondary infections rather than from the suppression of bone marrow function. If the infection can be checked the bone marrow is given a chance to recover. The agranulocytosis may be due to such causes as sulphonamides, mapharsen, gold salts, thiouracil, etc.

Actinomycosis. Hamilton and Kirkpatrick (40) successfully treated 2 cases of cervical actinomycosis with three-hourly intramuscular injections of penicillin, the dose varying from 25,000 to 33,000 units, and a total of between 5 and 6 million units being given. We treated a case of actinomycosis of the pleura, in which the organism was penicillin-sensitive *in vitro*, with intramuscular and intrapleural injections of penicillin, but the disease proved fatal in a few weeks.

Wounds and Burns. The low toxicity of penicillin together with its high bacteriostatic action make it pre-eminently suitable for local administration at the wound sites especially when combined with a sulphonamide (see p. 25).

Skin Diseases. Penicillin may be used as a cream, or a spray containing 500 units of penicillin per ml. It is of value in skin infections caused by staphylococci and streptococci. Impetigo and sycosis barbæ respond well to local treatment, but if cases relapse a course of intramuscular injections is useful in reaching the deeper parts of the hair follicles. Eczema with secondary infection is improved by penicillin locally. When the infection has cleared up it is important to treat the underlying eczema. Blepharitis responds well to penicillin cream. The spray or cream should be applied three or four times daily. The cream must be smeared on with a sterile spatula, or the handle of a spoon which has been boiled. Carbuncles, cellulitis, erysipelas and cavernous sinus thrombosis are best treated by intramuscular injection of 20,000 units every three hours for five days. Good results have followed the use of penicillin cream in pemphigus

neonatorum, but intramuscular injections are needed in severe cases.

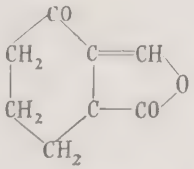
Eye Infections. Superficial eye infections caused by penicillin-sensitive organisms can be treated by instillation every one to two hours of drops containing 500–2,000 units of penicillin per ml., by the application of penicillin ointment, or by ophthalmic "Tabloids" containing 250 units. Blepharitis, conjunctivitis, ophthalmia neonatorum, infective keratitis and corneal ulcers respond well. Penicillin is especially of value in the treatment of sulphonamide-resistant staphylococcal infections.

Throat Infections. For streptococcal tonsillitis penicillin lozenges may be sucked every hour for 8 doses or the throat sprayed two-hourly with a solution containing 1,000 units per ml. In Vincent's infections it is usually necessary to give a course of intramuscular injections.

Other Antibiotics Isolated from Penicillium Moulds

Notatin. This metabolic product of Penicillium notatum favoured by acid media was isolated by Coulthard and co-workers (41) in 1942. The close similarity in properties between this material and the "Penatin" of Kocholaty (42) makes it possible that the two substances are identical. Notatin acts as an enzyme on the substrate glucose producing gluconic acid and hydrogen peroxide. By virtue of this hydrogen peroxide a powerful antibiotic action is displayed *in vitro* against many types of organisms. As this activity is lost in the presence of catalase, this material has little clinical value.

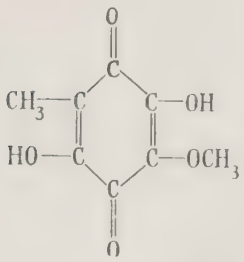
Patulin.



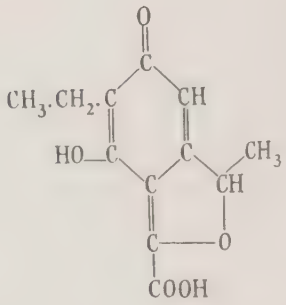
Indications at present suggest that patulin, from Penicillium patulum (43), claviformin, from Penicillium claviforme (44), and clavicin, from Aspergillus clavatus (45, 46), are all identical compounds. Evidence of similarity has been put forward by Chain et al. (47) and independently confirmed by Conn and

Geiger (48). Patulin is active against a number of Gram-positive and Gram-negative organisms *in vitro*, but unhappily this activity is not maintained *in vivo*. Clinical trials of patulin against the common cold have lent no support to the earlier claims that protection was afforded.

Penicillic Acid (γ keto β methoxy δ methylene Δ^{α} hexenoic acid). This antibiotic product of Penicillium puberulum was first isolated by Alsberg and Black in 1913 (49). Isolating the compound in quantity from an alternative source, Penicillium cyclopium, Raistrick et al. (50) established the constitution and demonstrated the activity of the compound against Gram-positive and Gram-negative organisms. It now appears that this antibiotic activity is only one phase of a general toxicity towards protoplasm, a toxicity which precludes penicillic acid from therapeutic use.



Spinulosin



Citrinin

Spinulosin and citrinin, products respectively of Penicillium spinulosum and Penicillium citrinum, display antibiotic activity *in vitro* by virtue of a general toxic action towards protoplasm. Both compounds possess a *p*-quinonoid structure which is known to be inimical to normal growth. The high toxicity of these compounds prohibits their clinical use.

Antibiotic Substances from Sources other than Penicillium Moulds

Aspergillic Acid ($C_{12}H_{20}O_2N_2$). This optically active crystalline compound is a product of the metabolism of Aspergillus flavus (51, 52). Although active *in vitro* against both Gram-positive and

Gram-negative organisms, high toxicity precludes it from systemic use.

Helvolic Acid ($C_{32}H_{44}O_8$). Following the report by Wilkins and Harris that culture filtrates from Aspergillus fumigatus mut. helvola possessed antibiotic activity, Florey et al. (53) isolated an optically active colourless crystalline mono-basic acid. Helvolic acid displays a limited bacteriostatic action against Gram-positive cocci *in vitro*, but at normal concentrations is not bactericidal. When the concentration of organisms is high, or when a substantial number of pus cells are present, even the bacteriostatic power is considerably reduced. Against experimental infection in mice, although the compound delays the onset of death by several days, it fails to give protection. Although not as toxic as aspergillic acid, helvolic acid is not suitable for clinical use.

Actinomycin A and B. These compounds were isolated by Waksman and Woodruff (54) from cultures of Actinomyces antibioticus. Both compounds have been obtained in a pure state, the former as a crystalline red pigment, the latter as an amorphous white powder. Actinomycin A is known to possess a quinonoid structure which may account both for its colour and bactericidal properties. Actinomycin B and the related product proctinomycin have not been fully investigated but are known to display antibiotic activity.

Streptothricin and Streptomycin. Waksman et al. (55) isolated two antibiotic compounds from artificial media supporting cultures of soil organisms of the genus actinomyces. The compounds streptothricin and streptomycin, isolated respectively from cultures of Actinomyces lavendulæ and Actinomyces griseus, are of special interest because, unlike penicillin and tyrothricin, they display a bacteriostatic effect against several Gram-negative organisms.

A close similarity exists between the two compounds, not only in the mode of extraction, but also in chemical properties. Streptothricin and streptomycin are basic compounds soluble in water and in dilute mineral acids, but insoluble in most organic solvents. Although precipitated by protein reagents, they do not give the normal protein colour reactions, nor are they inactivated by incubation with proteolytic enzymes. The systemic use of streptothricin cannot be recommended, but streptomycin

is sufficiently free of toxic properties to be administered by any of the normal routes. As a water-soluble substance, streptomycin gains ready access to most body tissues. Unfortunately this same property is responsible for its rapid elimination by the kidneys. As in the case of penicillin, a therapeutic concentration in the blood can only be maintained by continuous intravenous drip. Large doses may be given intramuscularly or subcutaneously with little or no evidence of toxicity.

Streptothricin *in vitro* shows activity against Escherichia coli, Brucella abortus, Salmonella paratyphi and other organisms of the colon-typhoid dysentery group. Unexpectedly the Clostridial group of organisms and some coccal strains show considerable resistance. Robinson et al. (56) have shown that mice infected by Salmonella schottmuleri, Escherichia coli and Bacillus shigalis can be protected by systemic injections of streptothricin. Attempts to confirm this finding have been attended by a high mortality amongst the experimental animals. Until more is known of the toxicity of pure streptothricin, application of the drug will be limited to local and oral administration.

Notwithstanding their similarity in chemical properties, streptothricin and streptomycin differ noticeably in bacterial specificity. The difference is quantitative rather than qualitative. Tested *in vitro* against Gram-positive and Gram-negative organisms, neither compound is consistently superior, although with few exceptions they inhibit similar types. Alike in action against the coli-typhoid dysentery group, streptomycin has the vital advantage of low toxicity. Furthermore, and this alone justifies its importance, streptomycin *in vitro* exercises an inhibitory effect upon the growth of the tubercle bacillus.

Fortuitously the antibiotic action of streptomycin against the tubercle bacillus is also exercised *in vivo*. Smith and McClosky (57), comparing the efficacy of streptomycin and the sulphone promanide against experimental tuberculosis in guinea-pigs, conclude that, although streptomycin is undoubtedly superior to the latter, the two drugs administered simultaneously exercise a valuable synergistic effect. When 65% of the control animals had died and four of the surviving controls were obviously ill and losing weight, all the treated animals were not only alive but had gained substantially in weight. The tuberculosis index

figures at autopsy were respectively 10 (controls), 4·1 (promanide), 1·9 (streptomycin) and 0·5 (promanide and streptomycin).

Preliminary clinical reports by Hinshaw and Feldman (58) are more equivocal. In 16 cases of pulmonary tuberculosis treated with streptomycin, X-ray examination showed progressive healing of recently formed lesions. There was no corresponding improvement in established cases of tuberculosis, showing fibro-caseous deposits. The sputum remained positive and healing lesions reappeared after the cessation of treatment. More hopeful results were obtained against tuberculous infections of the urogenital tract. Sterility of the urine established by treatment remained up to four months after the antibiotic agent had been withdrawn.

Strepticidine ($C_6H_{18}N_6O_4$), a well characterised base obtained by acid hydrolysis of streptomycin, is devoid of the antibiotic properties of the parent substance (59).

Tyrothricin (Gramicidin and Tyrocidin). In 1939 Dubos (60, 61) announced the isolation of an antibiotic substance, subsequently known as tyrothricin, from a culture of the soil organism Bacillus brevis grown on a 1% tryptone broth. Within one year of the original isolation, Dubos et al. (62) had not only obtained the substance free of protein but had established the fact that the original material was a mixture of two crystalline compounds, gramicidin and tyrocidin. These two compounds are similar in structure, both being built up of amino acids, but differing in molecular size. A provisional figure for the molecular weight of tyrocidin has been given as 2,534 ; this molecular weight is almost twice that of gramicidin. In contrast to penicillin, these compounds differ in two important respects. In the first place the solubility of the compounds in water is so low that clinically they are applied either as an aqueous emulsion or in alcoholic solution. This lyophobic character limits the diffusibility to such an extent that the material must be brought directly into contact with the invading organisms at the site of infection. The second point, the relative heat stability of the compounds, is an advantage in that suspensions or solutions may be sterilised in the conventional manner.

Most of the exploratory work has been carried out using tyrothricin, nevertheless *in vitro* experiments have shown that

this material exercised a potent bacteriostatic action against most strains of Gram-positive cocci, including members of the β-hæmolytic group of streptococci. Both compounds are active individually; gramicidin is apparently the more potent. Sulphonamide-resistant forms are inhibited and the bacteriostasis which is characteristic of these compounds is in no way modified by the presence of *p*-amino-benzoic acid. Unfortunately the promise of early work has not been fulfilled, the difficulty being one of application. Oral administration is out of the question, as both compounds are destroyed by the digestive secretions. The intravenous, intramuscular and subcutaneous injection of tyrothricin produces so marked a hæmolytic action that the clinical application of the drug by these routes is also precluded. Successful administration of tyrothricin has been limited to local use.

Applied locally in the treatment of ulcers, promising reports have been recorded from several sources such as the Mayo Clinic (63) and Rammelkamp and Keefer (64). Herrell (65) has reported successful applications against infection by streptococcus pyogenes. The use of tyrothricin as a preventive against infections of the nasal and pharyngeal cavities and as a means of sterilising the wound following mastoidectomy is also recommended.

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CHAPTER III

THE VITAMINS

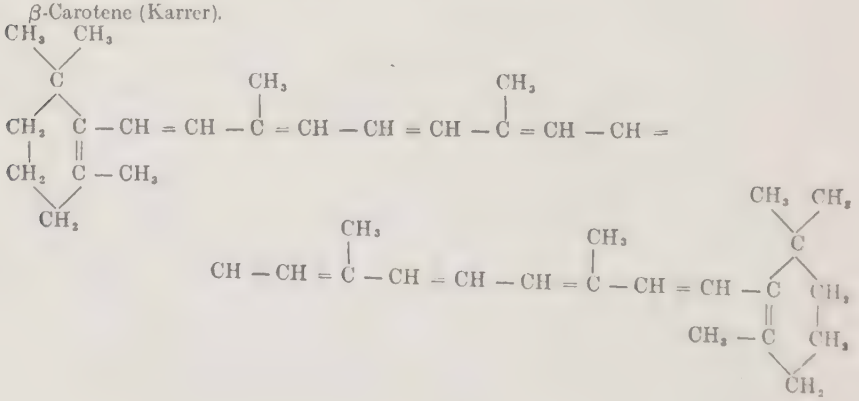
The actual date of discovery of the vitamins is difficult to place, since early travellers, even as far back as the Middle Ages, were aware that scurvy and similar diseases could be prevented only by feeding with fresh food, and more particularly fresh fruit. The matter received its first scientific establishment through the work of Sir Frederick Gowland Hopkins and Casimir Funk, who proved experimentally that animals could not exist on a diet composed of pure protein, fat, and carbohydrate. Hopkins showed that animals fed on such a diet not only failed to grow, but rapidly lost weight, and that this state of affairs could be immediately rectified by the addition of small quantities of milk to the diet, the quantity added being so small as to make no appreciable difference to the calorie intake. Since that time a great deal of work has been done on these accessory food factors, as Hopkins originally called them, and they have now been separated into a number of groups ; in all, there are to-day some thirty-one vitamins and provitamins which have either been definitely isolated or their presence proved by pharmacological experimentation. It is proposed now to describe those which are concerned with human nutrition :—

Vitamin A. This vitamin is present in fats and is soluble in fat solvents. It was discovered simultaneously in 1912 by McCollum and Osborn and Mendel. These observers showed that the liver of certain fishes such as the cod, and animal fats such as butter, contained a substance necessary for the growth of young animals. If animals are fed on a diet lacking this vitamin they suffer from a series of degenerative conditions, such as xerophthalmia, and succumb to intercurrent infections. Mellanby has maintained that this vitamin is responsible for raising the general level of resistance against infection, and so it has been termed by him the anti-infective vitamin. It has been shown

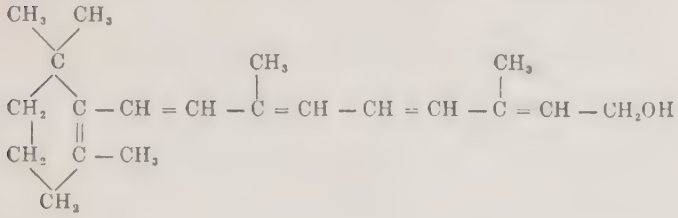
that a certain chemical test gives a rough indication of the amount of the vitamin which is present in a fat or oil. This test consists in treating the oil with a solution of antimony trichloride or arsenic trichloride, when a blue colour will develop. The amount of vitamin may be judged by the intensity of the blue colour. A quantitative estimation may be performed by estimating the colour by means of a Lovibond tintometer. The result is expressed as an arbitrary unit, which is known as the Lovibond unit if a 20% dilution of the oil in chloroform is used. The vitamin may also be estimated by a feeding test in which young rats are given a diet containing the optimum of protein, fat, carbohydrate and mineral salts and the other vitamins. The test is run on a series of animals, and as soon as they are losing weight steadily or are ceasing to grow, the unknown preparation is administered in varying quantities and the effect noted. The relationship between the chemical test and the physiological test is difficult to express, since it is highly probable that the efficacy of the vitamin in feeding tests is dependent upon a number of other factors which are at present uncontrolled.

As the administration of carotene to animals fed on a diet deficient in vitamin A confers all the benefits they would have received had the vitamin been present, it is now generally accepted that carotene is the main precursor of vitamin A, being converted in the body into vitamin A, and the international unit of vitamin A is taken as 0·6 γ of β carotene. From the chemical point of view the relationship is interesting, since carotene is a highly unsaturated hydrocarbon with the formula :—

β-Carotene (Karrer).



whilst vitamin A has the following formula : —



The vitamin has been synthesised and has a potency of 3 to 3·3 million international units per g. Vitamin A_2, which occurs in freshwater fish, differs only slightly from vitamin A in constitution.

Clinical Applications. Coward and Morgan (1) give the vitamin A content of foods in international units per g. or ml. as follows : Butter, average 60, egg yolk 30, carrots 19, cabbage 9, runner beans 6, milk 3 to 5. In 1937 a technical commission of the League of Nations decided that an adult requires about 3,000 international units of vitamin A daily. Children require about 4,000 international units and pregnant and nursing mothers 6,000 to 8,000 international units daily. A pint of milk, 1 egg, 1 oz. of butter and a helping of green vegetables will supply about 2,000 international units of vitamin A, and 1,000 international units of carotene. In war time the average adult only receives about 500 to 800 international units of preformed vitamin A daily. Carotene is less well absorbed from the alimentary tract in man than is vitamin A ; probably only 25% of carotene is absorbed. Carrots, spinach and broccoli tops are good sources of carotene, and with the present war-time allowance of milk and vitaminised margarine (which has only about half the vitamin A content of butter), $\frac{1}{2}$ lb. of carrots should be eaten weekly. They are best eaten cooked, as they are more easily digested and the carotene is not destroyed by cooking. It would appear that the diet of ordinary middle or lower class workers is deficient in vitamin A, and to this fact has been attributed the frequency of common ailments such as colds, bronchitis and septic complications, etc. The work of Mellanby has also indicated that lack of vitamin A may play a big part in the ætiology of puerperal sepsis. A severe degree of deficiency of vitamin A in the diet leads to xerophthalmia,

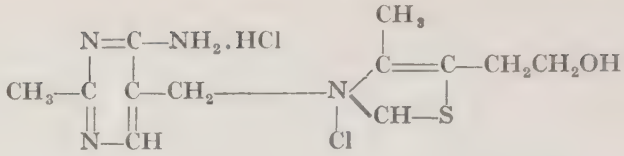
which was prevalent in Denmark in 1917, and is a common cause of blindness in India and China to-day. Further, striking evidence of relative hypovitaminosis A in apparently normal subjects is found by a study of the adaptation of the retina to light. It is now known that vitamin A is concerned in the regeneration of visual purple, the photo-sensitive substance of the retina which is responsible for rod vision. Investigations have shown that a large number of apparently healthy persons are affected with night-blindness in varying degrees, which may be tested by a special photometer. Thus Jeghers (2) testing 162 medical students at Boston found 55 deficient in vitamin A, as shown by this test. Harris and Abassy (3) reported similar results in school children attending elementary schools in London and Cambridge; 40 to 50% were normal, 20 to 30% slightly below normal, and 20 to 30% definitely deficient. A correlation was found between the diet and the dark-adaptation tests. "Among children having $\frac{2}{3}$ pint of milk at school 67% were normal on test, whereas of those having $\frac{1}{3}$ pint or less, only 37% were normal." Under war conditions in England, Yudkin (4) "found low adaptation in 9% of children at an urban institution, 15% among urban children from poor homes and 18% among some village children." It has been shown that the sensitivity to dim light may be greatly increased in these individuals by the administration of vitamin A. Yudkin (4) has shown that large doses are necessary to effect prolonged improvement, as much as 100,000 international units daily. A total dosage of 300,000 to 2,000,000 international units may be required, and the beneficial effect may last for weeks or months. It is usually given by the mouth in the form of one or other of the fish oils. At present the most concentrated form of natural oil is obtained from halibut liver. This may be administered either in the form of drops or in capsules. An alternative method of administration is to give the various concentrates, but it is stated by competent authorities that these are not so stable as the halibut liver oil. The preparation, Liq. vit. A. Conc. (B.P. Add.), contains 2,500 international units in 1 minim. Cod liver oil contains on an average 2,000 international units per g., and halibut liver oil 160,000 units per g. Thus a teaspoonful of cod liver oil contains 6,400 units, and a drop of halibut liver oil 3,200 units. When administered subcutaneously the preparation is said

to be not so active as when given orally. Although clinically the risk of hypervitaminosis A seems remote, Moore and Wang (5) have produced an experimental condition in rats which they attribute to massive oral doses of vitamin A. The symptoms, which include tenderness of the limbs and bone fractures, are absent if the vitamin is oxidised prior to administration. Post-mortem examination confirmed the presence of subcutaneous and intramuscular hæmorrhage, whilst X-ray photographs showed that the majority of the fractures occurred at the centre region of the long bones. Although the symptoms in many ways resembled those of scurvy, they did not respond to treatment with l-ascorbic acid or calciferol.

The Vitamin B Complex. Vitamin B was originally regarded as a single substance which was necessary for the maintenance of normal health in animals. It is now known to be a very complex mixture, the following constituents of which have been isolated and synthesised : aneurin, riboflavin, nicotinic acid, pyridoxin, biotin, folic acid, *p*-amino-benzoic acid and pantothenic acid. The last two compounds have not yet been proved to play an essential part in human nutrition.

Vitamin B_1. The absence of the B_1 factor is responsible for the peripheral neuritis of beri-beri, which is often accompanied by heart failure and gastro-intestinal disturbances. The first observations on this interesting substance were made by Eijkman in 1897. He showed, whilst working in a prison in Java, that fowls suffered from a type of polyneuritis similar to beri-beri, which at that time affected the prisoners with considerable severity. The fowls were fed on polished rice of the same variety as was given to the prisoners, and Eijkman showed that when the fowls were fed on unpolished rice the symptoms disappeared. A large scale experiment was carried out on the prisoners, and feeding a carefully controlled group on unpolished rice proved that the disease could be stamped out.

Vitamin B_1 is soluble in water. In 1932 Windaus (6) determined its composition to be $C_{12}H_{17}N_4OS$, and Windaus (7) and Williams (8), working independently, finally established the structure. The formula given below is that of aneurin, thiamine or vitamin B_1 chloride hydrochloride :—



The naturally-occurring vitamin is identical with the synthetic substance, both chemically and physiologically. The international unit of vitamin B_1 is defined as the anti-neuritic potency possessed by 3 γ of vitamin B_1 hydrochloride.

The assay of vitamin B_1 has been the subject of a number of publications which are summarised by Williams and Spies (9). The two main methods are the rat bradycardia test and a chemical test, known as the thiochrome method, whereby the vitamin may be estimated by measuring the intensity of fluorescence of one of its derivatives.

Considerable insight into the physiological function of aneurin has resulted from the discovery by Peters (10) that the oxygen uptake of brain tissue from avitaminose pigeons on a substrate of lactic acid or pyruvic acid is subnormal. When aneurin or aneurin pyrophosphate is added the respiration returns to normal and lactic and pyruvic acids disappear. The suggestion of Lohman and Schuster (11) that aneurin pyrophosphate is identical with co-carboxylase, the co-enzyme responsible for the metabolism of pyruvic acid, is now generally accepted.

Clinical Applications. The Scientific Adviser's Division, Ministry of Food (12) found that of 322 samples of National Wheatmeal Flour 81% of the samples contained more than 1·0 international unit per gram.

Harris and Wang (13) give the vitamin B_1 content of certain foods as follows, expressed in international units per gram : yeast extract 4·6 to 8·0, bacon 4, wheat germ 9·5, whole meal 0·87 to 1·7, oats 1·8, egg yolk 0·5, fresh milk 0·10 to 0·15. An adult requires about 700 international units daily, and 3 mg. of synthetic vitamin B_1 hydrochloride are equivalent to 1,000 international units. National Wheatmeal bread, made from 85% extraction flour, should supply sufficient vitamin B_1, and also nicotinamide.

For purposes of treatment vitamin B_1 can be administered by mouth in the form of Benerva tablets (3 mg.) t.i.d. or by intramuscular or intravenous injections of 5 to 25 mg. daily for 12 doses.

Children, pregnant and nursing women require a specially high vitamin B_1 intake, as do patients suffering from diabetes mellitus, increased thyroid activity and febrile diseases, and individuals undergoing prolonged muscular activity.

Alcoholic peripheral neuritis and other cases of neuritis of unknown origin may be due to deficient intake or absorption of the vitamin, and treatment in such cases with vitamin B_1 is worthy of trial.

Severe vitamin B_1 deficiency leading to beri-beri is rare in this country, but various complex and ill-defined conditions can be traced to partial deficiency. Early symptoms have been summarised by Williams and Spies (9) as follows : " Loss of appetite, weight, and strength ; muscle cramps ; diarrhœa, abdominal pain ; palpitation ; dyspnœa ; œdema ; burning sensations in various parts of the body ; vertigo ; headache ; cessation of menstruation ; numbness and tingling ; nervousness ; depression ; irritability ; distractibility ; apprehension and forgetfulness." We have found that in some cases in adults anorexia and flatulent dyspepsia have been improved by the administration of vitamin B_1. Sub-clinical deficiency in infants and children may be evidenced by constipation and a subnormal weight.

Heart failure, associated with chronic alcoholic poisoning, is in some cases due to vitamin B_1 deficiency. In these cases œdema may precede the onset of dyspnœa, and the face may be swollen as well as the legs. The vitamin deficiency may be demonstrated chemically by examination of the blood or the urine. The usual treatment for heart failure is not successful unless supplemented by injections of vitamin B_1. Attention was drawn to the condition by Aalsmeer and Wenckebach (14), and subsequently by Jones and Bramwell (15), by Bowe (16), and by Stannus (17).

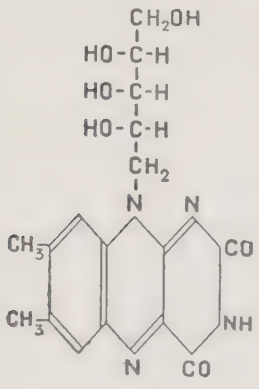
Williams, Shapiro and Bartelot (18), Forsyth (19) and Gretton-Watson (20) have reported on the treatment of acrodynia, or pink disease, by the administration of vitamin B_1. Very little is known of the causes of this condition, and the course varies widely, but it is well known that spontaneous and sudden recovery frequently occurs. Acrodynia in rats is associated with vitamin B_6 deficiency.

Wernicke (21) in 1881 described a syndrome characterised by oculo-motor palsies, mental changes and ataxia, resulting from acute hæmorrhagic polioencephalitis superior. Small degenerative

foci and varicosities are present in the periventricular grey matter. It is associated with chronic gastro-intestinal disorders, many cases being due to chronic alcoholic poisoning. It has subsequently been shown by Wortis et al. (22) that there is a disturbance of pyruvic acid metabolism, and in many cases improvement rapidly follows the administration of vitamin B_1, although it is not thought to represent a simple aneurin deficiency. A similar disease, known as chastek paralysis, can be produced in silver foxes by feeding them largely on raw fish. This can be prevented by adding aneurin to the diet, and it is possible that an anti-vitamin is present in raw fish, which inactivates aneurin.

Vitamin B_2 (Riboflavin). This is no longer regarded as the anti-pellagra vitamin, though its deficiency is considered to be responsible for some of the symptoms often associated with pellagra. Its constitution has been revealed through the work on the flavins. It has been known for many years that all plant and animal tissues contain a water-soluble yellow pigment known as riboflavin (lactoflavin) which exhibits blue fluorescence. This substance has been crystallised by many workers from various sources such as whey, egg-albumen, egg-yolk, liver, urine, etc.

Györgyi, Kuhn and Wagner-Jauregg (23) suggested in 1933 that vitamin B_2, an essential growth-factor for rats, was a flavin. They were able to show that for finely crystalline lactoflavin a dose of 7 to 10 γ constituted a rat unit. The Heidelberg group of workers proved that the synthetic riboflavin is both biologically and chemically identical with the naturally-occurring vitamin B_2. The formula of riboflavin is shown here. In rats the absence of vitamin B_2 from the diet causes cataract, stunting of growth, loss of hair and general lowering of condition.



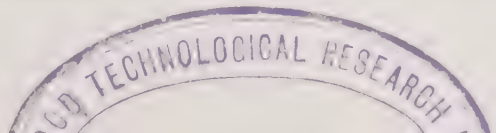
Riboflavin

The physiological function of riboflavin, like that of aneurin, is enzymic in character and is intimately associated with cell respiration. As a phosphoric acid ester, in association with a protein carrier, riboflavin has been identified with Warburg's "Yellow enzyme." The connection between this important respiratory function

and the symptoms of ariboflavinosis are not yet understood.

Clinical Applications. The chief source of vitamin B_2 is plant tissue, particularly young green leaves, but milk is a stable and dependable source containing 1 to 2 γ per ml. National flour contains 1·3 to 1·9 γ per g. and dried yeast 0·1 to 0·2 mg. per g.

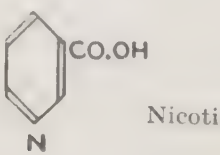
Stannus (24) first described the chief clinical features of riboflavin deficiency twenty years before the discovery of riboflavin. Sebrell, Butler, Wooley and Isbell (25) found, by study of the riboflavin balance in ten women, that the requirement of this vitamin is approximately 3 mg. per day. Lack of this vitamin in man produces a condition described by Sebrell and Butler (26) as ariboflavinosis. Eighteen women were given a diet with a low riboflavin content, and thirteen developed ariboflavinosis. The lips become red along the line of closure, and the mucous membrane is thin, shiny and denuded. This is known as cheilosis. Maceration and fissuring occur at the angles of the mouth, resembling the lesions known as perlèche, so-called from the verb *lécher*, as the patient licks the sore with the tip of the tongue. Seborrhœic accumulations are also noted at the naso-labial folds, on the alæ nasi, in the vestibule of the nose, and occasionally on the ears and eyelids. These lesions disappeared with the administration of 0·025 mg. riboflavin per kg. of body weight, and reappeared on discontinuing the vitamin. The tongue may also be affected, being purplish-red or magenta in colour, clean, with flattened or mushroom-shaped papillæ, and a burning sensation may be complained of. Further Kruse and his co-workers (27) have found that vitamin B_2 deficiency leads to keratitis and that the condition can be cured both in animals and in human subjects by the administration of the vitamin. Johnson and Eckardt (28) treated thirty-six patients suffering from rosacea keratitis with the vitamin, giving 3 mg. daily, and found that it will correct the condition when it arises from vitamin B_2 deficiency in the diet, but not when it arises in patients whose diet is not deficient but who show hypochlorhydria. Engorged limbic vessels are not a definite indication of ariboflavinosis. In order to diagnose the condition on ocular signs, new capillaries should be seen by slit-lamp microscopy, arising from the apices of the limbal loops and spreading on to the cornea, around the whole circumference and in each eye. Mann (29) has described a further corneal mani-



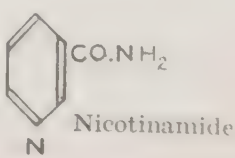
festation of ariboflavinosis, with marginal vascularities and opacities in the substantia propria. These changes disappear when riboflavin is administered. Jones et al. (30) described an outbreak of stomatitis due to riboflavin deficiency in a camp in North Africa when the daily riboflavin intake was reduced from 1·61 mg. to 1 mg. per head. It was quickly cured by giving 100 mg. riboflavin in five days, or by ½ oz. of fresh yeast daily. Partial syndromes of ariboflavinosis, without eye signs or tongue changes, have also been described by Duckworth (31), and in one case under our observation a patient complained of a sore hard palate ; the mucous membrane over the affected part was pale, and the symptoms were promptly relieved by the administration of 3 to 5 mg. of riboflavin daily, nicotinic acid and other remedies having failed. Relapse occurred when the treatment was omitted. Scarborough (32), describing some cases of circumcorneal injection as a sign of riboflavin deficiency, comments on this tendency to relapse in certain patients in whom an abnormally large amount of riboflavin appears to be required. Manson-Bahr (33) has suggested that vitamin B_2 deficiency (riboflavin and nicotinic acid) may be responsible for the glossitis found in pellagra, sprue, pernicious anæmia, the nutritional anæmias and idiopathic steatorrhœa. Stannus (34) suggests that the first tissue to feel the evil effect of riboflavin deficiency is the capillary endothelium.

Nicotinic Acid (Niacin). This is also known as the pellagra-preventing (P-P) factor. Pellagra is a deficiency disease prevalent in districts where maize is one of the staple foodstuffs, such as the southern states of the U.S.A., Rumania, Italy, Egypt and Southern Russia. Its symptoms are severe gastro-intestinal disturbances and wasting, associated with characteristic dermatitis ; in addition many subjects develop chronic dementia.

Experiments on dogs and rats, and later on human subjects, have shown that pellagra is essentially a deficiency disease which can be cured by the administration of nicotinic acid or its amide. Their formulæ are given below :—



Nicotinic Acid



Nicotinamide

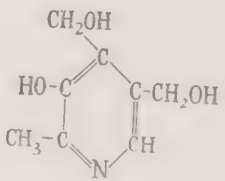
Nicotinic acid, like several other members of the vitamin B complex, is known as a widespread and important constituent of carbohydrogenase systems. Coenzyme I (adenosine-ribose diphosphoric-ribose nicotinic amide) plays the part of hydrogen carrier to enzyme systems which oxidise such substrates as alcohol, lactic acid and malic acid, whilst Coenzyme II (adenosine-ribose triphosphoric-ribose nicotinic amide) plays a similar *rôle* in the dehydrogenation of glucose, hexose monophosphate and glutamic acid. The reduced coenzymes are subsequently re-oxidised by transferring their acquired hydrogen to Warburg's "yellow enzyme." This important function has not yet been related to the clinical symptoms which characterise pellagra.

Clinical Applications. The distribution of nicotinic acid in foodstuffs has been determined by chemical and microbiological methods. Liver, kidney, pork flesh and yeast are good sources, whereas milk contains only small amounts. The daily requirement is 15 to 40 mg. From 30 to 50% of the ingested nicotinic acid is normally excreted in the urine as derivatives of nicotinic acid or as trigonelline. The blood level and urinary excretion of nicotinic acid in pellagrins does not differ markedly from the normal, using chemical methods of assay. It has been shown by Kark and Meiklejohn (35) that, contrary to the general view, porphyrins are not necessarily present in the highly pigmented urine of pellagrins. The blue fluorescence of the urine in these cases is attributed to the presence of a substance F_1 of unknown chemical composition. A second bluish fluorescent substance F_2, which is possibly thiochrome, is a normal constituent of urine and is diminished in quantity in the urine of pellagrins. Najjar and co-workers (36) found that the administration of nicotinic acid to pellagrins decreased the output of F_1, while raising the quantity of F_2. The minimum effective dosage is still not yet worked out either for the cure or prevention of pellagra, but Spies, Cooper and Blankenhorn (37) recommend 0·5 g. daily, given in five doses of 100 mg., as safe and effective in the usual case of pellagra. For parenteral injection they found that 50 to 80 mg. a day, in sterile physiologic solution of sodium chloride, were effective when injected intravenously. Sebrell (38) has emphasised that the best method of prevention is to bring about a permanent change in diet by adding as much green vegetables,

milk and lean meat as possible. Since it seems likely that vitamin B_1 plays some part in the development of the pellagra syndrome, the diet must provide for an adequate intake of this factor also.

King (39) suggests that Vincent's disease in man, in which there is ulceration of the mouth accompanied by the presence of fusiform bacilli and spirochætes, may be a pre-pellagrous condition. He records good results from the administration of nicotinic acid, 250 mg. by mouth in water, given daily for ten days. In glossitis and stomatitis due to nicotinic acid deficiency the tip and sides of the tongue are fiery red and swollen, and ulcers form on the tongue and buccal mucous membrane. Vincent's organisms may be found in the greyish exudate. On account of its vasodilator action intravenous injections of nicotinic acid (5 to 10 mg.) have been given in cases of cerebral thrombosis by Furtado (40), and in angina pectoris by Neuwahl (41), and in Ménière's syndrome by Atkinson (42), with promising results. Nicotinic acid may also be given by mouth in doses of 25 to 150 mg. daily, according to the flushing effect produced, in cases of intermittent claudication. In some cases the walking capacity of the patient is considerably improved. Gottlieb (43) described 5 cases of acute nicotinic acid deficiency, characterised by mental confusion or excitement, which were relieved by the administration of nicotinic acid in doses of 400 to 600 mg.

Vitamin B_6 (Pyridoxin). In 1934 Gyorgy (44), investigating the condition of acrodynia in rats, showed that, although the symptoms were associated with a dietary factor, none of the known members of the vitamin B complex afforded relief. In 1938 five independent groups of workers succeeded in crystallising the essential factor, for which the name " pyridoxin " was finally adopted. In the same year degradation and synthesis (45, 46) showed that pyridoxin was a pyridine derivative with the constitution shown :



Pyridoxin appears to be widespread in the animal and vegetable

kingdoms. Good sources are to be found in yeast, rice polishings and wheat germ, and although found in many other foodstuffs it is frequently associated with protein or starch, and, as such, is only assimilated after hydrolysis by cooking.

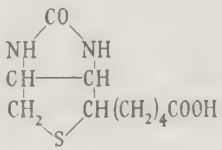
Whilst rats on being deprived of pyridoxin show loss of weight, œdema and dermatitis of the extremities, the corresponding condition in dogs and pigs is associated amongst other things with a disturbance of erythropoiesis. Independent workers (Chick et al. (47), Wintrobe et al. (48) and Borson and Mettier (49)) working variously on pigs and dogs, agree that the condition is one of hypochromic microcytic anæmia. The condition, although having elements of similarity with pernicious anæmia, does not respond to iron or liver extract therapy. On the contrary, as the symptoms intensify, there is a rise in serum iron which runs parallel to the anæmia. There is marked hypoplasia of the bone marrow and hæmosiderosis of the liver and spleen. Neurological disturbances of an epileptiform character also occur. At least in experimental animals, all these symptoms are relieved by the administration of pyridoxin. There is little doubt that much of the experimental work in the past has been complicated by the failure to recognise the part played by nicotinic acid and folic acid in related conditions.

Our clinical knowledge of pyridoxin is far from complete. The synthetic compound has been used in substantial quantities in the treatment of anæmias (Vilter, Schiro and Spies (50)), muscular atrophy and dystrophy (McBryde and Baker (51)), and Parkinsonism (Spies, Hightower and Hubbard (52)). The reports are conflicting and no well-defined clinical condition has yet been clearly associated with deficiency. The new information regarding the function of folic acid in macrocytic anæmia may help to resolve this problem.

Biotin. In 1927 Boas (53), showed that rats maintained on a raw egg-white diet developed symptoms of an eczema-like dermatitis which was associated with a loss of hair. Factors which protect rats from "egg-white injury" were isolated independently by Kogl and Tonnis (54) from egg-yolk, α-biotin, and by Du Vigneaud et al. (55) from liver concentrate, β-biotin, formerly known as vitamin H. Despite former assurances that these two compounds were identical, it now seems likely that they

are indeed distinct (56). The factor responsible for the symptoms of biotin deficiency has been isolated by Eakin, Snell and Williams and named Avidin (57). Avidin, which has been crystallised (58), is a protein-carbohydrate complex of molecular weight about 70,000. It forms a complex with biotin which renders the latter inactive.

The constitution of β-biotin has been elucidated and confirmed by the synthesis by Du Vigneaud and colleagues (59, 60) as :



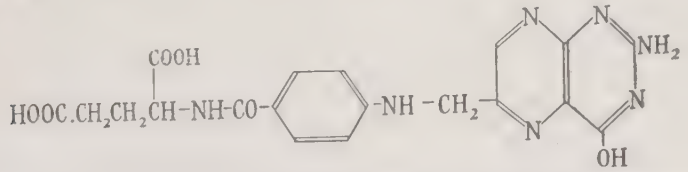
The natural vitamin is the *d*-compound and as the racemic form, tested on rats, has only half of the activity of the naturally-occurring material, it is questionable whether the *l*-form has any activity (Emmerson (61)), Ott (62)). According to Kogl et al. (63), the constitution of α-biotin differs significantly from that put forward by the American school, but as the compound has not yet been synthesised, no clinical significance is yet attached to it.

Biotin is found in close association with other members of the vitamin B complex, the chief sources being yeast, liver, eggs, peas and cereals. According to Sydenstricker et al. (64), the daily requirement of adult humans is about 150 μg.

The symptoms of biotin deficiency are not limited to rats. As an essential growth factor for many bacteria and moulds (Bios IIB), biotin has been known for over two decades, yet the recognition of its clinical importance is of very recent development. Experimental deficiency in monkeys may be induced by limiting the biotin intake, by adding raw egg-white to the diet, or by suppressing the synthesis of biotin by the intestinal flora by the administration of sulphonamides (Waisman et al. (65)). The syndrome in monkeys is characterised by a thinning of the fur, a loss of hair colour and by a conspicuous dermatitis of the face, legs and arms, all of which symptoms are relieved by the administration of 20 μg. of synthetic biotin daily. Symptoms of biotin deficiency in man described by Sydenstricker (64) include a scaly dermatitis, a greyish pallor of the skin, atrophy of the lingual

papillæ and disturbed erythropoiesis. A very remarkable case of clinical biotin deficiency has been quoted by Williams (66).

Folic Acid. The subdivision of the vitamin B complex is apparently so endless that it is a pleasant task to be able to report some simplification. With the purification of the various reported essential factors, it becomes increasingly probable that three factors hitherto treated as separate entities are, in fact, identical. These are (*a*) folic acid, the acid factor isolated by Williams et al. (67) from spinach, and found by him to be a growth-stimulant of the organism streptococcus lactus, (*b*) the " norit eluate factor " reported by Snell and Peterson (68) in yeast and liver, and found to be an essential factor for the growth of lactobacillus helveticus, and (*c*) vitamin B_c, a dietary factor described by Hogan and Parrott (69), the absence of which is responsible for the symptoms of macrocytic hypochromic anæmia in chicks. The earlier claim of Mitchell and Williams (70) that folic acid bore a close resemblance to xanthopterin, a pigment found in butterfly wings, seems to be inconsistent with the inability of this compound to relieve the characteristic symptoms of vitamin B_c deficiency in chicks. Notwithstanding this fact, the close similarity in physical and chemical properties, together with the stimulatory effect upon lactic acid organisms, held in common by these three factors, support the claim to identity (Pfiffner et al. (71)). Some clarification of this problem may be anticipated following the recent characterisation of the liver factor. Angier et al. (72) have shown by degradation and synthesis that this factor possesses the following structure :



N-[4-{[(2-amino-4-hydroxy-6-pteridyl) methyl] amino} benzoyl]glutamic acid.

The presence of glutamic acid and *p*-aminobenzoic acid residues in the molecule is interesting. Hitherto the pteridyl ring has not been encountered as such in the biological field.

The synthetic compound stimulates the growth of L. Helveticus (casei) and is active against anæmia in chicks. An analogous

synthetic compound prepared by omitting the glutamic acid residue fails to do either, but stimulates the growth of Streptococcus fæcalis R. Angier and co-workers also describe a second factor isolated from a fermentation mixture which, as it can be readily converted into the above compound, must be closely related to it.

Folic acid, as its name implies, is found in green leaves. Concentrates of the vitamin are usually obtained from spinach tops, yeast, or, if an animal source is preferred, from liver extracts. As this factor is almost certainly synthesised by the intestinal flora, it is difficult to estimate the daily requirements. Established deficiency in adults has been cured by the administration of 5 mg. per day for ten days.

Rats maintained on a diet supplemented with aneurin, riboflavin, pantothenic acid, pyridoxin, nicotinic acid, choline and biotin, but containing 1% sulphaguanidine, developed symptoms of agranulocytosis and leucopenia which responded promptly to the administration of crystalline folic acid (Daft and Sebrell (73)). Within four days, the white cell counts rose from 2,700 to 14,400 per cubic millimetre, with a simultaneous increase in the percentage of granulocytes from 1 to 40.

That folic acid not only stimulates the production of white cells, but also exerts a profound effect upon erythropoiesis is apparent from the clinical reports of Spies (74, 75) on the treatment of nutritional macrocytic anæmia. Out of 42 cases of macrocytic anæmia treated, 26 responded immediately to treatment with 20 mg. of synthetic crystalline folic acid administered either parenterally or orally. The cases, which included five of Addisonian pernicious anæmia as well as macrocytic anæmia of pregnancy and of sprue, showed a sharp reticulocyte response followed by an increase in red cell count and hæmoglobin content. Independent confirmation of the efficacy of folic acid in the treatment of sprue has been given by Darby, Jones and Johnson (76), who report the successful treatment of 3 classical cases by intramuscular injection of 15 mg. of folic acid.

Para-aminobenzoic Acid. This substance was first recognised as a vitamin by Ansbacher (77). He fed black and piebald rats on a diet which produced greying of the fur, a condition known as achromotrichia. When this diet was supplemented daily by 3 mg. of *p*-aminobenzoic acid, the animals grew normally pig-

mented fur within a month. Ansbacher (77) also showed that the vitamin improved the condition of chicks reared on a heated vitamin K-deficient diet, while Sure (78) found that *p*-aminobenzoic acid was an essential dietary factor for lactation and reproduction in the rat. It is evident that the above results require further confirmation, since some workers have failed to substantiate the claims of Ansbacher. The formula of *p*-aminobenzoic acid, which is one of the B-complex vitamins, is as follows :

The substance has aroused considerable interest in recent years because of its inhibitory effect on the activity of sulphanilamide. This inhibition, which was demonstrated by Woods and Fildes (79) *in vitro* and by Selbie (80) in mice infected with streptococcus hæmolyticus, led Fildes (81) to conclude that *p*-aminobenzoic acid is an essential metabolite for bacteria. The vitamin appears to be widespread in plants and animals and has been isolated from yeast, which has been found to contain 0·5 mg. per 100 g. Little is known of the effect of a deficiency of the vitamin in human subjects or of the normal daily requirement. Sieve (82), however, claimed that *p*-aminobenzoic acid caused darkening of the hair in thirty cases of human achromotrichia.

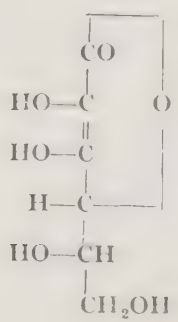
Microbiological Assay of Members of the Vitamin B Complex. In no single sphere of biochemistry have the methods of microbiological assay been more successful than in the estimation of vitamin B factors. As with more complex animals, selected strains of bacteria are apparently very discriminating in regard to the composition of the medium upon which they grow. Although organisms will fail to grow on a medium lacking in one essential factor, yet, over a limited range of concentration, they respond in an almost linear manner to addition of micro-quantities of the missing factor. It is upon this principle that the method of microbiological assay rests. Factors essential to bacteria include most members of the vitamin B complex and an impressive list

of amino acids, including all of those essential to man. By using pure solutions to produce standard graphs of growth against added factor, it has been possible to estimate, with a greater degree of accuracy than is possible by chemical methods, the concentration of these factors in foodstuffs.

The method will doubtless lend itself to the assay of these factors in biological materials. In most assays it has been possible to circumvent the technical difficulty of measuring the growth of bacterial cultures by using lactic acid producing organisms, and the linear relation between lactic acid production and the concentration of the missing factor has been demonstrated in many cases.

Phycomyces Blakesleanus has been used for a number of years in the determination of aneurin (Schopfer and Jung (83)). Less well known, but not less accurate, is the use of Lactobacillus Helveticus (L. casei ϵ) for the estimation of riboflavin (Snell and Strong (84)), pyridoxin (Carpenter and Strong (85)), biotin (Snell, Hutchings and Peterson (86)) and folic acid (Snell and Peterson (68)). For the estimation of nicotinic acid Lactobacillus Arabinosus is the organism of choice (Barton-Wright (87), Kent-Jones and Meiklejohn (88)). Other organisms which have been employed in similar assays include Neurospora Sitophila (Stokes et al. (89)), Streptococcus lactis (Snell, Guirard and Williams (90)) and Leuconostoc Mesenteroides (Barton-Wright et al. (91)). The latter is also recommended for amino acid assays.

Vitamin C. This substance, which is water-soluble, is responsible for protection against scurvy. It is found distributed fairly widely in animal tissues and also in the juice of fresh fruit, particularly citrus fruits. It has been shown to be *l*-ascorbic acid which has the constitution shown below :—



The compound is a powerful reducing agent and is unstable in alkaline solution. It is estimated biologically by its ability to protect guinea-pigs from scurvy, and chemically by titration with phenol-indo-2 : 6-dichloro-phenol.

Clinical Applications. The vitamin is administered in the prophylactic and curative treatment of scurvy, usually in the form of fresh orange juice. Orange juice contains 0·2 to 0·9 mg. per ml., rose-hip syrup 0·4 to 2·2 mg. per ml., blackcurrant purée 0·66 to 1 mg. per ml., blackcurrant syrup 0·63 mg. per ml., spinach 0·9 mg. per g., human milk 0·06 mg., and cow's milk 0·02 mg. per ml. The vitamin C content of cow's milk is lowered by pasteurisation, by standing, by further heating and by dilution.

Vegetables should be cut up only just before cooking, and dropped in small quantities into boiling water, care being taken that the water does not go " off the boil." Further, if vegetables are not eaten as soon as cooked, the ascorbic acid gradually deteriorates. The daily requirement of vitamin C for an adult is 50 to 100 mg., for a child 50 to 150 mg., and for an infant 15 to 50 mg. Alternatively it may be given as tablets of ascorbic acid each containing 50 mg. of crystalline vitamin C, 1 to 3, t.d.s., or in adult scurvy, ascorbic acid mg. 100 to 300 may be administered subcutaneously, t.i.d. There is no danger of hypervitaminosis. It has been suggested that many chronic illnesses may be due to a degree of vitamin C subnutrition (latent scurvy). Abbasy, Hill and Harris (92) have shown that in active rheumatism the ascorbic acid excretion in the urine is below normal, and they conclude that in the infection which underlies rheumatic fever there is a greatly increased metabolic use of, and need for, vitamin C, and a correspondingly lower degree of saturation of the body reserves. Kaiser (93), however, determining the vitamin C content of the blood in normal and rheumatic children, found no striking difference and considered that vitamin C is probably not a significant ætiological factor in rheumatic infections. Thus children may show a fragility of capillaries, especially in the early spring. Campbell and Cook (94) found that gingivitis, characterised by painful and bleeding gums, rapidly healed if ascorbic acid was given in doses of 300 mg. daily for four days. Glazebrook and Thomson (95) made observations on youths between the age of fifteen and twenty years who were having a diet in which the

vitamin C was largely destroyed by the methods of cooking and distribution; 335 had ascorbic acid administered daily for several months. None of them developed pneumonia or rheumatic fever, whereas in a group of 1,100 controls there were 17 cases of pneumonia and 16 of acute rheumatism. Lack of healing of wounds (Bourne (96)) and of gastric ulcers in patients undergoing treatment has been ascribed to deficiency of vitamin C in the diet. In some cases of pernicious anæmia a satisfactory response to liver therapy is not obtained until ascorbic acid is also administered. Harris (97) concludes that the addition of extra vitamin C to wartime diets has increased resistance to infections, has promoted healing of wounds, and has improved weight and height gains in children.

The diagnosis of vitamin C subnutrition may be carried out as follows: Harris and his co-workers (98) have studied the concentration of ascorbic acid in the urine both before and after the administration of test doses of ascorbic acid to human subjects. They found that normal individuals excrete significant amounts of ascorbic acid in their urine, usually over 13 mg. daily. The vitamin is derived from the diet. If the ascorbic acid is withheld from the diet, ascorbic acid is excreted at a steady rate for a time, indicating the existence of appreciable reserve stores of the vitamin. In general, the urinary excretion reaches an equilibrium level proportional to the dietary intake of the vitamin. Harris regards a daily excretion below 13 mg. as suggestive of vitamin C deficiency. The effect of a test dose of 700 mg. of ascorbic acid per 10 stones of body-weight depends on the state of nutrition of the subject with respect to vitamin C. In normal individuals there is a sharp increase in the excretion of ascorbic acid, usually on the first day following the test dose. A patient is regarded as " unsaturated " if the excretion rate does not show a rapid increase above the resting level. When dealing with patients with a low reserve of ascorbic acid a better idea of the degree of unsaturation may be obtained if the test dose is administered for several consecutive days and the daily output of vitamin recorded.

The chief difficulty of the test is due to the instability of ascorbic acid, which oxidises rapidly in air. For this reason, the urine should be collected in dark stoppered bottles and treated with

one-tenth of its volume of glacial acetic acid. Analysis should be performed at once. The night urine should be collected in the same way and despatched to the laboratory for analysis early in the morning. The whole of the urine passed in twenty-four hours must be collected. Unless these precautions are taken, loss of ascorbic acid will occur and may lead to an erroneous conclusion. If the output is under 13 mg. per day a deficiency of vitamin C may exist. For the test dose, about 700 mg. of ascorbic acid should be given for every 10 stones of body weight, and the urine collected for the following two or three days. If a small response results, the dose should be repeated on one or two further days in order to distinguish between the milder degrees of deprivation and more severe hypovitaminosis.

Harris and Abbasy (99) have proposed a modified test over a shorter period, suitable when the collection of full twenty-four-hourly specimens may be inconvenient. At 9 a.m. the patient is instructed to urinate. This specimen is discarded. The patient is then instructed not to urinate until 12 noon if possible, when the test specimen representing one-eighth of the total day's excretion is obtained and analysed. The same programme is repeated on a second and third day in order to establish the resting level. After this, the test dose of 700 mg. of ascorbic acid per 10 stones of body-weight is administered on one or more further days. In these cases the urine samples are collected during the same afternoon over a three-hourly period, *e.g.*, 2 to 5 p.m., and analysed at once.

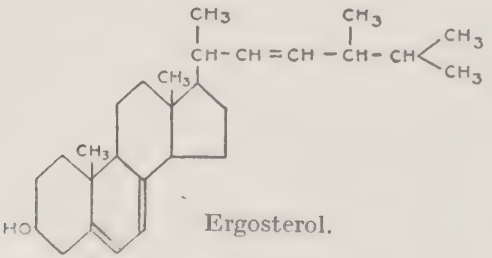
Investigation of the ascorbic acid content of the blood has indicated the existence of a threshold value of about 1 mg. per 100 ml. of plasma. Lower thresholds are sometimes observed. Normally, the effect of a test dose is to increase the plasma ascorbic acid over the threshold value, with the appearance of the vitamin in the urine. Ungley (100) showed that with deficiency of vitamin C the resting level of ascorbic acid in the plasma is low, and is only slightly increased by the dose. However, the plasma value, although dependent on the intake of ascorbic acid in the diet, is not a good index of a significant deficiency of the vitamin. Thus, Lund and Crandon (101) in a study of a human volunteer found that the plasma level of ascorbic acid dropped to zero in six weeks, whereas clinical scurvy took five months to develop in a subject

placed on a diet free from ascorbic acid. It was also found that the reducing substance in the " buffy coat " of centrifuged blood, presumably consisting of ascorbic acid, took four months to drop to zero and thus provided a more reliable index to the danger of clinical scurvy.

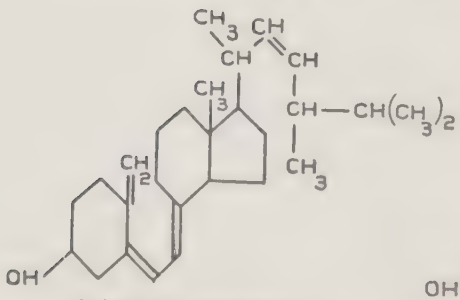
Vitamin D. This anti-rachitic vitamin is found in fish liver oils, but can also be prepared by the irradiation of inactive sterols found in nature. Thus, the irradiation of ergosterol leads to the formation of calciferol which was formerly thought to be the naturally-occurring vitamin. The constitution of the naturally-occurring vitamin is not certain, since many sterols on irradiation produce active compounds. At least ten provitamins D are known, the four listed below having been isolated in the pure state :—

Vitamin D	*Provitamin D*
D_2 . . .	Ergosterol
D_3 . . .	7-dehydrocholesterol
D_4 . . .	22-dehydroergosterol
D_5 . . .	7-dehydro-sitosterol

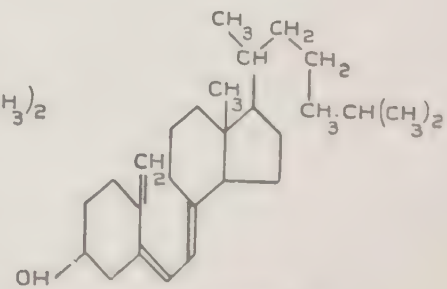
The compound D_3, which has been isolated from tunny fish oil, has a high activity when tested in chickens and it is suggested that this is the naturally-occurring vitamin D. The formulæ for ergosterol, calciferol and vitamin D_3 are as follows :—



Ergosterol.



Calciferol (vitamin D_2).



Vitamin D_3.

The method of standardising vitamin D consists in employing the same type of method as already recounted for the other vitamins. There is, however, considerable difference in that the great importance of the presence of this vitamin has succeeded in producing a definite quantitative method of estimation. Rats are fed on a special diet containing no vitamin D until rickets develops. The stock is then divided into groups, and to each group graded doses of the preparation are given by mouth. To another series a standard preparation of vitamin D is given, and by carefully grading the doses attempts are made to produce the same effect as with the standard preparation, and by this means a definition of the activity of the unknown in terms of the standard may be arrived at. The actual technique of determining the degree of healing of the rickets varies considerably. For example, X-ray photographs may be taken of the leg bones, when uncalcified areas can readily be seen. An alternative method is that known as the "line test." In this, animals are killed and sections of the long bones are made and the epiphyses are stained with silver nitrate. The preparation is then exposed to the light, with the result that at the epiphyseal line a black area is produced. By this means the degree of healing may be studied. It is thus possible to obtain the potency of the unknown in terms of the standard. The international unit is $\frac{1}{10000}$ mg. of irradiated ergosterol. The D vitamins are stable to cooking.

Clinical Applications. The vitamin D content of certain foods, according to Coward and Morgan (1), is as follows, expressed as international units per g.: Egg yolk 1·5 to 5, cream 0·5, butter 0·4 to 4, milk 0 to 0·1, cod liver oil 480 units per teaspoonful, and halibut liver oil 48 units per drop. Fresh herrings contain 250 international units per oz. The use of vitamin D is very wide, and it is usually given either in the form of cod liver oil, halibut liver oil, or some similar preparation, or, alternatively, as irradiated ergosterol. As a prophylactic against rickets, most foods given to children are reinforced either by the addition of irradiated ergosterol or by the irradiation of the actual product; infants usually require 700 to 1,500 international units daily, and as a curative dose 3,000 to 5,000 international units daily. The British Pædiatric Association recommends 700 international units daily for a baby during the first year. This is contained in 8 drops

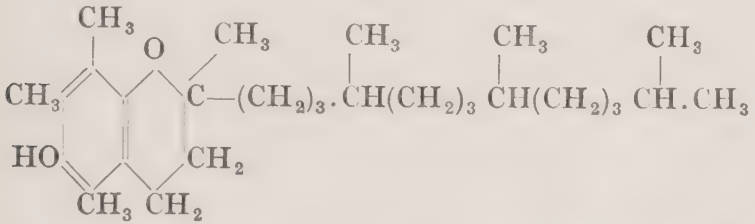
of Radiostoleum, 10 drops of halibut liver oil, 12 drops of Adexolin, 280 drops of cod liver oil compound, and 560 drops of 50% emulsion of cod liver oil (2 drops =1 minim). Liq. vit. D Conc. (B.P. Add.) contains 1,500 international units in 3 minims. Ten thousand units daily would constitute an overdosage, resulting in increased density of bone, fever, etc. Massive doses of vitamin D, in the form of irradiated ergosterol (300,000 international units or more daily) have been used to raise the blood calcium in tetany, and in the treatment of lupus vulgaris, rheumatoid arthritis and hay fever. Such doses are not devoid of danger. Tumulty and Howard (102) record the case of a man given 750,000 international units daily for twelve days to aid the union of a fracture. His blood calcium rose to 15 mg. per 100 ml., the serum phosphorus not falling below 3·8 mg. per 100 ml. He was seized with nausea, abdominal pains and severe vomiting, associated with renal failure. The vitamin D had been administered in milk, in which vehicle it is ten times more potent than in oil.

Dihydrotachysterol (A.T. 10), although possessing only slight antirachitic potency, has also been employed to raise the level of blood calcium in idiopathic and post-operative (hypoparathyroid) tetany. It appears to possess no special advantage over calciferol for this purpose. Albright (103) recommends that the urine of patients under treatment with massive doses of calciferol or A.T. 10 should be regularly tested for calcium by the Sulkowitch reagent. This reagent consists of 2·5 g. oxalic acid, 2·5 g. ammonium oxalate and 5 ml. of glacial acetic acid dissolved in water to make 150 ml. One volume of urine is treated with 1 volume of reagent. If no precipitate of calcium oxalate is obtained there is little calcium present in the urine and the serum calcium level is probably from 5 to 7·5 mg. per 100 ml. If there is a fine white cloud the level of the calcium in the serum is in the satisfactory range. If the mixture looks like milk, the danger of hypercalcæmia is present. No method has been worked out for the actual isolation of vitamin D from the urine, and therefore we have no definite evidence of a sub-rickets condition.

Vitamin E. It has been shown that animals fed on diets deficient in certain vegetable substances can no longer reproduce. The fœtus becomes resorbed following placental changes after

the twelfth day. In the male this deficiency leads to destruction and atrophy of the testes. The distribution of vitamin E in nature is widespread, cereal grain, green leaves, legumes and nuts being good plant sources of the material. As far as is known, wheat germ oil is the richest source, and most workers attempting to obtain the pure vitamin from natural sources have used this as a starting material. Vitamin E is classified among the fat-soluble vitamins, and is destroyed by atmospheric oxygen in the presence of alkali.

Three substances, α-, β- and γ-tocopherol have been isolated and found to possess vitamin E activity. α-tocopherol has been synthesised by Karrer et al. (104) and Todd et al. (105), and is as active as the most potent vitamin E concentrates. The formula of α-tocopherol is shown below :



β- and γ-tocopherol possess one methyl group less than α-tocopherol and exhibit only half the activity of α-tocopherol when tested on rats.

Clinical Applications. Vitamin E is put up in 3 m. capsules (Viteolin), each containing the unsaponifiable matter of 5 g. wheat germ oil, or as α-tocopherol tablets, 3 mg. (Ephynal). Fresh dried whole-wheat germ may also be given by mouth, ½ oz. twice daily. One international unit corresponds with 1 mg. of the acetate.

Sterility. Good results have been claimed by some authors such as Currie (106) in the treatment of habitual and threatened abortion by the administration of Vitamin E in the form of wheat germ oil extract 3 m. capsules, one capsule daily for three to six months up to the onset of labour. It is difficult, however, to obtain adequate controls, and some authorities are sceptical of the results claimed. Drummond (107) says that in human beings the process of normal pregnancy, like that in the goat and unlike that in the rat, may not be dependent on vitamin E.

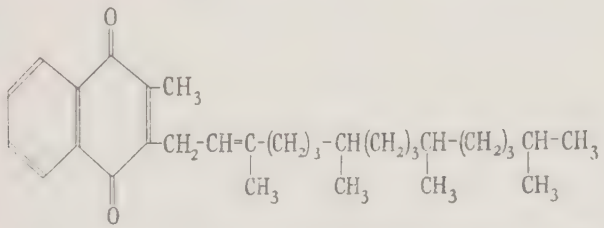
Nervous and Muscular Lesions. Bicknell (108) suggests that vitamin E may be deficient in the normal diet of man and that the nervous and muscular systems may suffer in cases of hereditary disposition or from toxic influences such as syphilis. He also suggests that the anti-sterility factor, α-tocopherol, is not identical with the myotropic and neurotropic factors, although all are present in wheat germ oil. He treated cases of muscular dystrophy in children and amyotrophic lateral sclerosis and tabes dorsalis in adults with fresh dried whole-wheat germ, ½ oz. twice daily for a period up to eighteen months. He obtained good results with the myopathies and came to the conclusion that they are deficiency diseases and curable. Demole (109) considers that only one factor, α-tocopherol, is needed to prevent paralysis in experimental animals deficient in vitamin E. Wechsler (110) obtained good results in two cases of amyotrophic lateral sclerosis treated with pure synthetic α-tocopherol in the form of Ephynal tablets, 3 mg., 2 to 3 t.d.s. Subsequent work by various investigators, such as Sheldon et al. (111), Doyle and Merritt (112), Denker and Scheinman (113), Ferrebee et al. (114), and Fitzgerald and McArdle (115) indicate that vitamin E lack does not cause muscular dystrophies or motor-neurone disorders in man. Further, administration of vitamin E, either in the crude form or as the synthetic preparation, alone or combined with vitamin B_6, does not cure such disorders. This opinion is based both on clinical observations, and on the estimation of urinary creatine. The excretion of the latter results from nutritional muscular disturbances, and, if the patient is on a creatine-free diet, its excretion should diminish as the muscular disease improves. Bicknell and Prescott (116) criticise these adverse findings on the efficacy of vitamin E, and say that in many cases enormous doses were administered which may act adversely by actually causing a fall in the vitamin E content of the blood. They further say that in many of the unsuccessful cases the disease had lasted for many years, when recovery of muscular function could hardly be expected.

Vitamin K. The history of vitamin K, the "Koagulations-vitamin," dates back to the suggestion of Dam (117) made in 1934, that the spontaneous hæmorrhages found in newly-born chicks were the result of the absence of a fat-soluble factor from

the diet. These hæmorrhages, although closely resembling those of scurvy, did not respond to the administration of l-ascorbic acid, and, furthermore, differed from those of avitaminosis C in that they were associated with hypoprothrombinæmia and a prolonged blood clotting time (Schonheyder (118), Dam et al. (119)). The analogous clinical conditions in man were recognised by Brinkhous et al. (120) in 1937 as hæmorrhagic disease of the new-born and as a hæmorrhagic tendency in cases of biliary obstruction.

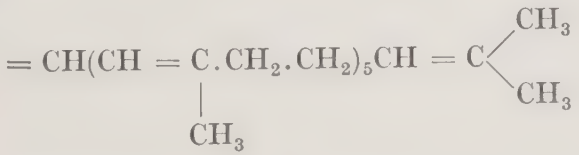
Although fat-soluble, vitamin K differs from vitamins A and D in that it predominates in plant sources. Doisy and his co-workers (121) isolated two crystalline compounds, vitamin K_1 (alfalfa) and vitamin K_2 (putrefied fish meal) which, although similar in physiological activity, differed in detail in their chemical composition.

Vitamin K_1 has been shown to be 2-methyl-3-phytyl-1 : 4-naphthoquinone and is represented by the following formula :—



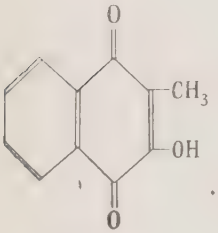
2-methyl-3-phytyl-1 : 4-naphthoquinone (vitamin K_1).

Vitamin K_2 (122), the second of the naturally-occurring vitamins, differs in that the phytyl group in the 3-position is replaced by

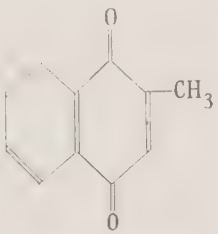


The molecular structure is not very specific and many synthetic 1 : 4 naphthoquinone derivatives display activity. An intact benzene ring and methyl group in the 2-position have been found in all active compounds to date. Not only can the group in the 3-position be replaced by hydrogen without loss of activity, but the quinonoid oxygens may also be replaced by a variety of

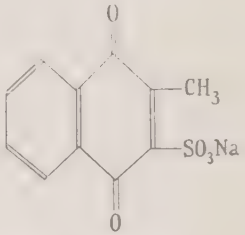
groups. Phthiocol (2-methyl-3-hydroxy-1 : 4-naphthoquinone) was the forerunner of a series of compounds in which lyophilic groups (— OH, SO_3H, etc.) were deliberately introduced to endow the synthetic drugs with water-solubility. Typical water-soluble vitamin K substitutes such as sodium 2-methyl-1 : 4-naphthoquinone-3 sulphonic acid, and 2-methyl-1 : 4-naphthoquinone bisulphite (Stewart (123)), are readily absorbed even in the absence of bile secretion and are suitable for oral administration (Smith and Owen (124)). The fat-soluble analogues are usually administered intramuscularly in an oily medium or orally accompanied by bile salts to aid absorption.



Phthiocol



Menaphthone



Sodium 2-methyl-1 : 4-naphthoquinone-3-sulphonic acid.

Some doubt exists as to the site and mode of action of vitamin K. The older school of thought maintained that the " Koagulations-vitamin " played no direct part in the coagulation of blood, but was only associated with prothrombin formation. Ample evidence is forthcoming (Fitzgerald and Webster (125), Kark and Souter (126)) that some four hours after the administration of vitamin K to patients suffering from hypoprothrombinæmia there is a distinct rise in the blood prothrombin concentration and a corresponding fall in prothrombin time, but as to whether vitamin K is an essential unit in prothrombin, or whether it stimulates the formation of prothrombin in the liver, no opinion was expressed. Lyons (127) has produced definite evidence which bears on this problem. According to the classical theory of Morawitz, supported by Mellanby (128), thrombin reacts with, or combines with, fibrinogen to form the insoluble fibrin clot. Lyons succeeded in dividing thrombin into two active components, Thrombin A and B, neither of which alone will convert fibrinogen into fibrin, although a combination of both will do so.

Thrombin A reacts with fibrinogen to form an intermediate compound, fibrinogen B, between fibrinogen and fibrin. Fibrinogen B can be converted into a normal fibrin clot by the addition of Thrombin B or a minute quantity of 2-methyl-1 : 4-naphthoquinone (vitamin K).

Lyons suggests, therefore, that either vitamin K is the prosthetic group of the enzyme thrombin, or that vitamin K along with a protein carrier acts as an oxido-reductase system. Support to the former theory is given by the fact that the products of tryptic digest of thrombin give colour reactions characteristic of 1 : 4-naphthoquinones.

Within a few days of birth, the danger of hæmorrhagic disease of the new-born recedes and with the establishment of an intestinal flora the child becomes less dependent upon an exogenous source of vitamin K. As this synthesis and absorption from the intestine persists throughout adult life, it is difficult to get an accurate idea of normal human requirements. Sells, Walker and Owen (129) place the requirements of the new-born at 1 to 2 μg. per day. Figures given by other workers are substantially the same.

In vitamin K therapy synthetic substitutes have largely replaced concentrates from natural sources. Phthiocol (2-methyl-3-hydroxy-1 : 4-naphthoquinone), the first synthetic analogue, no longer finds as widespread use as Menaphthone (2-methyl-1 : 4-naphthoquinone) which is put up in capsules of 1 to 10 mg. for oral administration and in oil for intramuscular injection. For more rapid control of hæmorrhage, intravenous injection of water-soluble derivatives is recommended (Almquist and Klose (130)). The prompt response in these circumstances, however, is not maintained, and a prolonged rise in prothrombin level is best achieved by intramuscular injection of an oil-soluble derivative.

Clinical Applications. Various preparations are available for clinical use. Klotogen is an oily concentrate of the naturally-occurring vitamin. It is put in capsules for oral administration. Kapilon is an oily solution of a synthetic analogue of vitamin K. It is put up in 1 ml. ampoules containing 5 mg. of the pure substance, for intramuscular injection, and in tablets of 10 mg., and in " liquid " containing 10 mg. in 1 ml. for oral administration. Prokayvit is a similar synthetic substance put up in 1 ml. ampoules each containing 5 mg. for intramuscular injection. The synthetic

substances have the advantage of being considerably cheaper than the naturally-occurring vitamin. Bilein capsules each contain gr. 5 bile salts.

The chief indications for the use of vitamin K are :—

1. *Spontaneous subcutaneous and internal hæmorrhages, or the liability to bleeding during and after operation.* The predisposing condition here is the absence of bile from the intestinal tract, resulting in deficient absorption of the vitamin. The most important causes are obstructive jaundice, biliary fistula, and less often acute hepatosis and yellow fever. Investigations have shown that vitamin K is a fat-soluble vitamin which cannot be absorbed from the intestine in the absence of bile-salts. The hæmorrhagic tendency which results is due to a low plasma prothrombin level. The method for estimating this is described on p. 376, and this appears to be the best guide to the need for vitamin K therapy. If vitamin K is given by mouth in the absence of bile from the intestinal tract, bile-salts must also be administered in order to ensure its absorption from the intestine. This is not necessary if the vitamin is injected. In some cases, however, absorption appears to be slow after intramuscular injection. In cases of operation for obstructive jaundice, Klotogen, 1 capsule, or Kapilon, 1 tablet, and Bilein, two gr. 5 capsules, are given t.i.d. with meals for at least four days before operation and for three to four days subsequently. Successful results have been obtained by Macfie et al. (131) in cases of obstructive jaundice using intramuscular injections of the synthetic vitamin K. Thus Kapilon or Prokayvit, 1 to 2 ml., may be given daily by intramuscular injection for two to three days prior to the operation, and 1 ml. subsequently for two to three days. In many cases of obstructive jaundice in which the prothrombin level of the blood is low, there is a failure of response to vitamin K. This is probably due to extensive liver damage. In such cases, as shown by Kark and Souter (132), if the vitamin fails to raise the prothrombin level, the only alternative method of treatment is blood transfusion.

2. *Hæmorrhagic disease of the newborn.* In newborn infants it is common to find slight hæmorrhagic tendencies during the first week, but occasionally the hæmorrhages are so severe as to cause death. The investigations of Dam et al. (133) indicated that the cause of the hæmorrhage is vitamin K deficiency developing a few

days after birth, and ceasing usually after the eighth day. They have found that in one case the administration of the vitamin together with bile-salts through a stomach tube raised the prothrombin level to normal within forty-one hours. A low prothrombin level, however, is probably not the sole cause of the hæmorrhage, as the level may be low without bleeding occurring. Subsequent investigations in America by Sandford et al. (134) have thrown some doubt on the matter. Although the administration of vitamin K to newborn babies lessened the normal fall in the prothrombin level during the first three days of life, hæmorrhagic manifestations occurred as frequently in the babies thus treated as in the untreated. These, however, were all minor hæmorrhages, and not those characteristic of hæmorrhagic disease of the newborn. They further showed that the administration of vitamin K to the mother before delivery will raise the plasma prothrombin value of the infant. Norris and Bennett (135) determined the prothrombin value of the mother and of the newborn child, and showed that as there appears to be no relationship between the two, it is unlikely that the prothrombin passes directly from the mother to the fœtus, and it may be formed in the placenta. It does not seem possible to decide by an examination of the maternal blood, whether or not vitamin K should be given to the mother before the birth of the child. In addition to the pathological hypoprothrombinæmia, the clotting time of the blood is prolonged in the hæmorrhagic disease of the newborn. The hypoprothrombinæmia occurring in icterus gravis neonatorum, anæmia neonatorum and hydrops congenitus also responds to the administration of vitamin K.

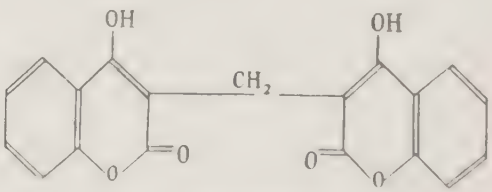
The prophylactic treatment of neonatal hæmorrhage consists in giving the mother Kapilon 5 tablets or $1\frac{1}{2}$ drams liquid by mouth twelve to four hours before the baby is born, and the baby is given 36 drops (18 m.) by mouth on the first day of its life. Lehmann (136), in Sweden, using a vitamin-K analogue, sodium-2-methyl-1, 4-naphthohydroquinone disulphate, which is completely soluble in water and absorbed without the presence of bile salts, treated 13,250 full term infants with doses of 1 mg. (20 drops of a 0·1% solution) by mouth within an hour of birth, the results being controlled by prothrombin estimations. In comparison with 17,740 untreated cases, Lehmann concluded

that death from bleeding was reduced by 1·6 per 1,000 in the treated infants, and recommends the method for general prophylaxis. The curative treatment consists in the intramuscular injection of a preparation such as Kapilon, 1 ml., repeated daily. In severe cases blood transfusions should be given.

The question as to whether vitamin K deficiency may arise through some dietary lack in otherwise normal subjects cannot yet be answered. A study by Kark and Lozner (137) of four non-jaundiced subjects with diminished prothrombin levels suggests that there may be a dietary deficiency of vitamin K in man, but it seems likely that such a condition can only arise under abnormal circumstances and in conjunction with other nutritional deficiencies. A low plasma prothrombin level may occasionally be responsible for hæmoptysis in pulmonary tuberculosis. We have had one such case under our care, and Sheely (138) reports four cases of hæmoptysis associated with prothrombin deficiency in which the administration of vitamin K appeared to be a likely factor in controlling the bleeding.

Readers are referred for further consideration of this subject to the monograph by Butt and Snell (139).

Dicoumarol. No account of the relationship of vitamin K deficiency to hypoprothrombinæmia would be complete without reference to the hæmorrhagic symptoms which characterise sweet clover disease in cattle, as described by Roderick (140) and Quick (141). The cause of this condition has been shown by the Wisconsin school to be due to the action of a substance present in the sweet clover diet. The active principle, isolated and synthesised by Link et al. in 1941 (142), is Dicoumarol (3 : 3-methylene-bis-4-hydroxycoumarin) :



Dicoumarol

Krumer and Barker (143) and Lucia and Aggeler (144) showed that, administered orally, this compound induces hypopro-

thrombinæmia inside seventy-two hours unless considerable amounts of vitamin K analogue are administered simultaneously. The fact that the prolonged clotting time in hæmorrhagic disease is always associated with a fall in blood prothrombin level, together with the relatively slow recovery following protection by vitamin K therapy, suggests that dicoumarol inhibits the production of prothrombin by the liver. Link et al. (145) have shown that in rats with pronounced hepatic disorder, recovery is very slow even when ample vitamin K is administered.

Clinical Applications. For the prevention of post-operative thrombosis, pulmonary embolism, and as a prophylactic in thrombophlebitis, dicoumarol has advantages over heparin. Apart from considerations of cheapness, it is effective orally and exercises a more prolonged action. The dangers of spontaneous hæmorrhage are never absent, however, and the course of treatment with dicoumarol must always be controlled by daily determinations of clotting time.

The drug is put up in mg. 50 tablets, called Dicoumarin. Allen, Barker and Waugh (146) gave to their patients 200 to 300 mg. by mouth on the first day and 200 mg. on the second day, and subsequently it was administered in doses sufficient to keep the prothrombin time between thirty-five and sixty seconds (normal fifteen to twenty seconds). This effect should be maintained for about ten days after pulmonary embolism, or after the onset of acute thrombophlebitis. It should not be used for patients who are bleeding and it is particularly dangerous in subacute bacterial endocarditis. Further, it should not be used in cases in which there are ulcerating lesions, renal disease, obstructive jaundice, severe liver damage or high fever. Zucker (147) reported the successful use of dicoumarol in cavernous sinus thrombosis. Davis and Porter (148) recommend the daily administration of three separate doses of 100 mg. every eight hours in the treatment of puerperal thrombosis. There is usually a delay of twenty-four hours after the first dose before the effect is produced, and this may persist for two to ten days after the dicoumarol is discontinued. Signs of overdosage may occur if the prothrombin time is over sixty seconds. They include lassitude, aching in the costo-vertebral joints, bleeding into the skin, joints, brain, viscera or from the gums. A blood transfusion, using blood not more than

four days old, will restore the prothrombin time to normal. Vitamin K is useless for this purpose.

Vitamin P. Szent-Györgyi and co-workers (149) isolated from paprika and lemon juice a substance which, whilst capable of controlling the petechial hæmorrhages which characterise certain diseases such as vascular purpura, was not l-ascorbic acid. Chemical investigation of the material showed that it was an equilibrium mixture of two flavanone glucosides, hesperidin and its chalcone, eriodictin, but, as concentrates of the vitamin have been produced by Bacharach and Coates (150) which, when tested by these workers, were many times more active than crystalline hesperidin, the identity of the essential factor is by no means established beyond doubt.

Clinical Applications. Scarborough (151) considered that Szent-Györgyi's cases were unsuitable for the demonstration of a new vitamin and that the only suitable cases for investigation are those of known low vitamin intake, many of which show capillary fragility unrelated to vitamin C deficiency. Scarborough (152) says that as the result of nutritional deficiency and vitamin P lack, petechial hæmorrhages occur in the skin with much decreased capillary resistance. The patient complains of lassitude with pains in the shoulders and legs, but there are no hæmatological changes. These symptoms are relieved by the administration of vitamin P but not by vitamin C. Scarborough (153) recorded two cases of purpura senilis in which the capillary resistance was low and associated with a tendency to bruise. Administration of Permidin apparently increased the capillary resistance and controlled the tendency to bleeding. Permidin is available in tablet form for clinical use. Each tablet contains the equivalent of 0·25 g. of hesperidin, the usual dose being 1 to 4 tablets daily. Kugelmass (154) treated successfully two cases of allergic purpura in girls, characterised by fever, abdominal pain, bloody stools and purpura. He prepared the vitamin P from orange peel. The solution contained 50 mg. per ml. of flavones consisting of eriodictyol glucoside and hesperidin and the dose was 150 mg. orally. Vitamin P does not appear to be efficacious in the treatment of purpura hæmorrhagica, or of mechanical purpura.

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CHAPTER IV

GLYCOSURIA AND DIABETES MELLITUS

The historical aspects of diabetes mellitus, the theories of carbohydrate metabolism, and the discovery and mode of action of insulin, have all been summarised so frequently that no attempt will be made here to reconsider these questions. The following references will be found to cover the above points : Banting and Best (1), the discovery of insulin ; Collip (2), Doisy et al. (3), Dudley (4 and 5), Dodds and Dickens (6), Burn (7), Dodds and Dickens (8), covering the general chemical and physiological properties of insulin.

The following factors influence carbohydrate metabolism and sugar excretion :—

1. The intake and absorption of carbohydrate.
2. The action of the liver, kidneys, pancreas, thyroid, pituitary and suprarenals.
3. The nervous factor.

Failure or variation of any of these factors may produce glycosuria, and it will, perhaps, be as well to summarise briefly the mechanism of each.

Alimentary Glycosuria

In 1916 Taylor and Hulton (9) stated they were unable to induce alimentary glycosuria by the ingestion of large amounts of glucose. MacLean (10), in 1924, also cast some doubt upon the ease with which this condition could be produced. Working with Bennett, one of us (E. C. D.) (11) repeated these experiments, and found that even 500 g. of glucosum liquidum (B.P.) were unable to produce glycosuria in the majority of normal men, and it was concluded that the upper limits of carbohydrate tolerance are governed merely by the subject's appetite, and that any attempts to demonstrate increased carbohydrate tolerance by alimentary tests are useless.

Hepatic Glycosuria

There is no incontrovertible evidence in favour of a hepatic glycosuria as a clinical entity.

Renal Glycosuria

The normal kidney will not let sugar pass into the urine unless the concentration in the blood is over 180 mg. per 100 ml. This is known as the leak point of the kidney. In the condition under discussion, the leak point is lower than the normal, hence sugar escapes into the urine although the blood sugar is below 180 mg. per 100 ml. There are varying degrees of renal glycosuria, according to how far the leak point is removed from the normal.

As can be seen, this condition is characterised by glycosuria in the presence of a low or normal blood sugar. Renal glycosuria is usually regarded as an innocent condition requiring no treatment, but it is possible that in some cases it may be followed by the development of real diabetes. For this reason it is advisable for blood sugar tolerance estimations to be made from time to time. In other cases, as described by Macpherson (12), spontaneous hypoglycæmic attacks may occur, probably due to associated hyperinsulinism.

Pancreatic and Endocrine Glycosuria

Here the condition is due to the absence of the internal secretion of the pancreas, which enables the tissues to utilise glucose. According to Bensley and Lane (13), the cells found in the islets are of two varieties, α and β, according to whether their granules are fixed respectively by alcoholic or aqueous media. The β cells are stated to be affected in diabetes mellitus, since they are seen to contain no granules. This inability to utilise carbohydrate is interpreted by the body as a stimulus for the production of more glucose, with the result that all available carbohydrate is poured out into the blood, producing a hyperglycæmia, and, since the tissues cannot utilise the sugar, it is excreted in the urine.

Although the above explanation can be accepted as a working clinical hypothesis, research on the pharmacological action of

insulin tends to show that the pancreas plays a much more important and complicated role.

When all the available carbohydrate has been thrown into the circulation, some proteins are converted into glucose and are excreted. The fat metabolism is also affected, producing a condition of acidosis and ketosis.

In hyperthyroidism, glycosuria is fairly common, whilst in certain pituitary lesions it is also observed.

An injection of adrenaline also gives rise to glycosuria. The action of this hormone lies in the fact that it stimulates the liver to break down glycogen, with the result that hyperglycæmia and glycosuria ensue. Adrenaline, therefore, appears to antagonise the action of insulin. It is known experimentally that adrenaline will help to abolish insulin convulsions. Pituitrin also inhibits the action of insulin.

Nervous Influences. Diabetic piqûre

By stabbing the floor of the fourth ventricle, sugar can be made to appear in the urine, and this is preceded by hyperglycæmia. This effect cannot be produced if the splanchnic nerves are cut. The explanation is that the diabetic puncture causes a stimulus to the suprarenals *viâ* the cord and splanchnic nerves, with the result that adrenaline is discharged into the circulation. This causes the hepatic glycogen to be broken down to glucose, as in the case of adrenaline glycosuria. Although this form of glycosuria is mainly of theoretical interest, cases occur where pressure of cerebral tumours, a cerebral hæmorrhage or meningitis, etc., cause the appearance of sugar in the urine.

Chemical Examinations Necessary in Cases of Suspected Glycosuria

Examination of the Urine

Qualitative Test for Sugar

Benedict's test [1] is the most suitable for general use. To carry out the test, 5 ml. of the reagent are poured into a test tube,

[1] The qualitative reagent is prepared as follows :—
173 g. of crystallised sodium citrate and 100 g. of anhydrous sodium carbonate (or 200 g. crystallised) are dissolved in 700 ml. of water. 17·3 g. of fine crystallised copper sulphate are dissolved in 100 ml. of water. The copper sulphate solution is poured into the former solution, and the volume is made up to 1,000 ml.

and 8 drops of urine are added. The contents of the tube are boiled over the flame for two minutes and then allowed to cool. If a large number of tests are to be made, the tubes may be set up in a rack and immersed in a boiling water bath for five minutes. The technique described above must be rigidly adhered to, since the actual quantities of urine, reagent, and time of heating were all worked out after a careful series of experiments. Any departure from it will rob the test of its specificity. If glucose is present, the reagent will change colour from clear blue to an opalescent green, or, if a large quantity of sugar is present in the urine, the final colour will be an opaque red. It is possible to obtain some idea of the amount of sugar present in the specimen by a study of the colour of the contents of the test tube. The following scheme will indicate the kinds of reduction given by urines containing quantities of sugar between 0·1% and 2% :—

0·1% greenish opalescence ; no red colour ; no precipitate.

0·2% as above, but slight yellowish precipitate ; fluid blue on standing.

0·3% green colour, with slight orange precipitate ; fluid blue on standing.

0·5% definite orange precipitate ; fluid still blue on standing.

1·0% heavy orange-brown precipitate ; still some blue colour in fluid on standing.

2·0% bright red, heavy precipitate ; blue colour of fluid almost disappeared.

Benedict's reagent is reduced by glucose, lævulose, lactose, pentose and homogentisic acid, but not by uric acid and creatinine ; hence a positive Benedict's test implies some derangement of carbohydrate metabolism, with the exception of homogentisic acid. In the majority of cases a positive Benedict's test means glycosuria, yet the possibility of lactosuria and pentosuria should never be lost sight of. The former condition occurs in pregnant and lactating women, whilst the latter is a rare disorder of metabolism, to some extent simulating diabetes mellitus. There are many ways of identifying what particular sugar is causing the reduction. Thus some workers prefer to prepare osazones and identify the sugar by the microscopic appearance of its phenyl-hydrazone, whilst pentoses can be differentiated from other urinary sugars by observing what

deflection is caused on the plane of polarised light and by special tests, such as that of Bial.[1] Glucose and lactose are dextro-rotatory, whilst urinary pentoses are without action on polarised light. These methods, although accurate, require apparatus and special skill, and hence are unsuited to general practice. The best reaction for clinical purposes is the fermentation test. A little yeast is ground up with the suspected urine until a thin paste is formed, and a fermentation tube is filled with the mixture. A urine definitely known not to contain sugar and one to which glucose has been added are treated similarly, and the three tubes are set up in a warm place overnight. In the morning they are inspected, and, if sugar is present in the suspected urine, the top of the fermentation tube will contain gas, as will also the tube containing the positive control. There must be no gas in the tube containing the urine with no sugar. It must be emphasised that the positive and negative controls form part of the test, and must never be omitted, since it is possible to have a yeast that will not ferment glucose, or on the other hand, one which will produce gas when no glucose is present.

Having ascertained the presence of glucose with certainty, one proceeds to the tests for acetone bodies.

It must be realised that these tests are of much greater importance than the percentage of sugar, since the presence of acetone bodies in large amounts means that the patient is in imminent danger of coma. Every patient whose urine contains large quantities of these bodies should be kept under close observation.

Tests for Acetone Bodies

Aceto-acetic Acid

Gerhardt's Test. This test consists in adding 10% ferric chloride, drop by drop, to the urine. At first a precipitate of ferric phosphate appears, which usually redissolves in excess of the reagent, owing to its acidity. If aceto-acetic acid is present in concentration of 0·07% or over, the solution turns a Bordeaux red colour. If any doubt is felt upon the genuineness of the colour,

[1] *Bial's test for pentose.* 5 ml. of Bial's reagent (1·5 g. orcinol, 500 g. fuming HCl, 20–30 drops of 10% $FeCl_3$ solution) are boiled in a test tube, and 2–3 ml. of urine are added. The heating is discontinued, and if pentoses are present, the solution turns green.

it should be compared with that given by ferric chloride added to normal urine. If a positive reaction by this test is given, an alteration must be made in the treatment, since a severe degree of ketosis is present. On the other hand, a negative reaction does not signify that the patient is entirely free from the dangers of ketosis. The reason for this is two-fold : firstly, the relative insensibility of the reaction ; and secondly, the fact that urinary aceto-acetic acid is converted on standing into acetone, which does not give Gerhardt's test.

Gerhardt's test is not without fallacies. Thus the presence of any phenolic drug in the urine will give a colour reaction with ferric chloride somewhat similar to that obtained with aceto-acetic acid.

If the patient has been taking drugs, such as aspirin, salicylic acid or salicylates and their derivatives, misleading results may be obtained. The colour, however, is quite different from that given by aceto-acetic acid, since it is much darker and of a more violet hue. Although this difference can be easily detected with practice, it is a good plan to boil a specimen of the urine prior to the test. If the colour is due to aceto-acetic acid, a negative reaction will be given on the addition of ferric chloride, whilst in the case of phenyl derivatives the colour will not be affected. This is due to the fact that aceto-acetic acid is volatile, whilst the other compounds are not.

Rothera's Test for Acetone and Aceto-acetic Acid.[1] This reaction is by far the most satisfactory test for ketosis. It is carried out by saturating about 20 ml. of urine with ammonium sulphate, by shaking with the crystals in a test tube. Two or three drops of concentrated ammonia, and a few drops of a freshly prepared dilute solution of sodium nitroprusside in water, are added, and the tube is shaken.

If the reaction is positive, a delicate permanganate tinge develops, which gradually deepens. A brown colour does not constitute a positive reaction. The amount of aceto-acetic acid can be judged by the depth of colour and the rapidity with which it develops. Thus Kennaway (14) points out that Rothera's test can be classified as follows :—

[1] It is essential that this test is performed in a laboratory that is not used for blood analysis, otherwise contamination of the nitrogen-free blood reagents will occur and inaccuracies will result.

1. A quick, strong reaction corresponding to 0·25% aceto-acetic acid.

2. A slow, weak reaction corresponding to 0·0005% aceto-acetic acid.

Reactions intermediate between these two extremes indicate proportional concentrations of aceto-acetic acid.

A faintly positive Rothera's test is not of such grave importance as a positive Gerhardt's reaction, owing to the much greater delicacy of the former.

There are no fallacies in Rothera's test, and it should be performed on every diabetic urine.

Estimation of Sugar in Diabetic Urine

Polarimetric Methods

These are suitable for hospital work, where large numbers of estimations have to be performed. The methods are very accurate, and most of the objections are mainly theoretical. Thus, since certain bodies, such as β-oxy-butyric acid, are lævo-rotatory, they would tend to reduce the dextro-rotatory power of the urine. The quantity of these bodies is, however, very small, and their

Table showing the Excretion of Sugar in various Representative Cases

Case.	Sugar. %	Volume of urine in 24 hours. ml.	Total amount of sugar in 24 hours. g.
Mild diabetes mellitus .	0·5	1,800	9
Severe diabetes mellitus .	9	5,500	495
Renal glycosuria . .	0·2	1,200	2·4
Thyroid glycosuria . .	1	1,300	13

specific index of rotation is negligible when compared with the dextro-rotatory powers of glucose. Before taking a polarimetric observation, it is usual to clarify the urine by some procedure. If 10 ml. of 10% basic lead acetate solution are added to 40 ml.

of urine, the filtrate obtained after shaking is quite suitable for observation.

Blood Examinations in Cases of Glycosuria

The Dextrose Tolerance Test

The patient comes to the laboratory in the morning fasting from the night before. He should take a standard amount of carbohydrate (300 g.) in the diet for the previous three days, so that subsequent tests may be comparable. Blood is collected and the patient is given 50 g. of dextrose, dissolved in 300 to 400 ml. of water, to drink. Blood is collected at half-hourly intervals for two hours, thus making four specimens after the dextrose and one before. The sugar content of each is estimated by the method described in the chapter on blood analysis.

The following table gives typical findings in various conditions :—

Blood Sugar Content in mg. per 100 *ml.*

Case.	Before Dextrose	½ hour after.	1 hour after.	1½ hours after.	2 hours after.
Normal man .	100	120	130	110	100
" Lag " Curve .	100	150	230	140	120
Mild diabetes .	170	187	198	190	182
Severe diabetes .	240	270	294	300	314
Renal glycosuria	98	100	94	96	98

Normally there is a rise in sugar content following the meal, but never above 150 mg. per 100 ml. The sugar content is back to normal again in two hours. Himsworth (15) showed that the readings in the dextrose tolerance test vary with alteration in the diet consumed by the patient during the preceding week. Thus the tolerance is low with a low carbohydrate, high fat diet, and it is supranormal with a high carbohydrate, low fat diet, as shown in the table below. With a low carbohydrate, high fat diet a diabetic curve can be obtained in health. The change in the

tolerance depends solely on the carbohydrate in the diet; the greater the carbohydrate the greater is the sensitivity of the patient to the insulin secreted by his pancreas.

Blood Sugar Content in mg. per 100 *ml.*

Diet.	Before Dextrose.	½ hour after.	1 hour after.	1½ hours after.	2 hours after.
C. 50 g. F. 240 g. .	100	170	200	160	145
C. 500 g. F. 40 g. .	100	118	118	110	120

Fraser et al. (16) advise that the patient be given at least 300 g. of carbohydrate for three days prior to the test, as then the blood sugar level becomes much more responsive to insulin, probably because less glycotropic hormone is secreted from the pituitary. In some cases the blood sugar rises sharply to above 180 mg. per 100 ml., but returns to normal within the two hours. This is called a " lag " curve, and indicates that the sugar-storage mechanism cannot keep pace with the dextrose absorption, and there is therefore a storage lag indicated by the rapid rise above the upper normal limit. It occurs often after gastro-enterostomy and in some cases of duodenal ulcer owing to the rapid stomach emptying and the consequent rapid absorption of dextrose from the intestine. Clinically there is a symptomless glycosuria when the patient is taking a normal diet. Although formerly regarded as an early stage in the production of diabetes mellitus, it is now thought that there is no abnormality in carbohydrate metabolism. There appears no reason therefore either to restrict the diet or to reject the patient for life insurance. In the mild diabetic the resting blood sugar is higher, and after the administration of dextrose the content rises above the leak point of the kidney, 180 mg. per 100 ml., and the return to normal is not so sharp. In the severe diabetic this condition is accentuated, and there may be no attempt at a return to the previous level during the period of the test.

Renal glycosuria is characterised by a subnormal blood sugar content throughout the experiment.

The Insulin Tolerance Test

The changes in insulin sensitivity in health with variations in diet, as judged by the insulin depression curve following the intravenous injection of insulin, were described in 1935 by Himsworth (15). The insulin tolerance test is used to determine the sensitivity of the blood glucose level to insulin, and its responsiveness to the hypoglycæmia resulting from the injection of insulin. The patient should take 300 g. of carbohydrate by mouth daily for three days before the test. After twelve hours without food the fasting blood sugar is determined and then 0·1 unit of insulin per kg. of the ideal body weight (as determined from height-age tables) is injected intravenously. Specimens of blood are then taken at 20, 30, 45, 60, 90 and 120 minutes and the blood sugar determined. 0·01 ml. of 1 in 1,000 adrenaline per kg. of body weight is then injected intramuscularly and further blood sugar determinations made 45 and 60 minutes later. Blood pressure readings are made when the blood is taken and any hypoglycæmic symptoms are noted. In severe cases of pan-hypopituitarism, Addison's disease or hyperinsulinism it may be necessary to terminate the test by giving dextrose, either by mouth or intravenously, owing to the gravity of the hypoglycæmic symptoms. The following results are based on the paper by Fraser et al. (16).

Blood Sugar per cent. of Fasting Level

Case	0 min.	20 min.	30 min.	45 min.	60 min.	90 min.	120 min.
Normal . . .	100	46	48	70	80	95	115
Addison's disease .	100	70	55	50	58	40	—
Pan-hypopituitarism .	100	52	40	42	42	60	60
Anorexia nervosa .	100	55	60	92	88	90	85
Primary myxœdema .	100	80	70	55	60	78	80

The blood sugar figures are charted as a percentage of the fasting blood sugar so as to render the results comparable. Thus a fasting blood sugar of 70 mg. per 100 ml. is charted as 100%,

and the subsequent readings are expressed as corresponding percentages.

Normally the blood sugar falls to about 50% of its fasting level in the 20 to 30 minute samples and is back to the fasting level in the 90 to 120 minute samples. In insulin-resistant cases the fall never reaches 50% of the fasting level, or does not do so for 45 minutes or longer. In hypoglycæmia unresponsiveness there is an absent or delayed rise. In Addison's disease there is deficient sugar production from protein, and the test shows hypoglycæmia unresponsiveness. In pan-hypopituitarism there is hypoglycæmia unresponsiveness due to lack of the corticotropic hormone, and also of the glycotropic hormone. There is a 50% fall in the blood sugar level in 20 or 30 minutes, and at the end of 120 minutes the blood sugar is less than 90% of the fasting level. A case of the pituitary type of myxœdema with a characteristic insulin tolerance curve is described by Lerman and Stebbins (17). In anorexia nervosa similar changes are found, but to a less marked degree. In primary myxœdema there is a diminished response to insulin, whereas in myxœdema secondary to pan-hypopituitarism the changes are as described for pan-hypopituitarism. Cases of pan-hypopituitarism (anterior) showing the typical insulin tolerance curves have also been described by Fraser and Smith (18).

The Dextrose Insulin Tolerance Test

Falta (19) drew attention to the fact, which has been the experience of most practitioners called upon to treat patients suffering from diabetes mellitus, that in certain cases it is very difficult to obtain a satisfactory response to insulin. Thus arose the idea that there are two types of diabetes, the insulin sensitive and the insulin resistant. Himsworth (20) says that they may be distinguished by the dextrose insulin tolerance test. In both types there is hyperglycæmia unresponsiveness after giving dextrose by mouth, as shown by a dextrose tolerance test; in one due to lack of insulin, in the other due not to lack of insulin, but to insulin resistance. The test is performed by placing the patient on a fixed diet for a few days and then giving dextrose by mouth and simultaneously injecting insulin intravenously. The amount of insulin injected should balance the dextrose, so that

in a normal individual the blood sugar curve remains flat. Himsworth recommends 30 g. of dextrose and 5 units of insulin per square metre of body surface. Blood samples are collected at 0, 5, 10, 20, 30, 40, 50, 60, 75 and 90 minutes. In a case of diabetes mellitus which is insulin sensitive the curve is flat or shows an increased response to insulin, whereas in an insulin resistant case the curve approximates to that obtained with a dextrose tolerance test. de Wesselow and Griffiths (21) failed to confirm these findings, but it has been objected that their patients were not placed on a preliminary standard diet, and blood samples were only taken at 30 and 60 minutes.

Ketosis and Acidosis in Diabetes Mellitus

Up to the present the only reference to this condition has been the description of the tests for acetone bodies in the urine. These are by far the most important, and, owing to their simplicity and accuracy, must remain as the most suitable clinical tests for this condition.

The whole question of ketosis is intimately bound up with that of acidosis; consequently it is necessary to enter into the physiology of neutrality regulation. Space does not allow of a full treatment of this subject.

The hydrogen ion concentration of the blood under physiological conditions is always kept constant. It is only in the terminal stages of disease that any alteration occurs, and this usually precedes death. The constancy of the hydrogen ion concentration is the more surprising when one considers the number of factors which are tending to alter it.

Thus there is the constant production of acid from metabolic processes and muscular action. Again, large quantities of acid are poured off the blood during gastric secretion, and large quantities of alkali during pancreatic secretion. It is obvious that the regulating mechanism must be wonderfully efficient to keep the reaction of the blood constant during gastric secretion, since the HCl secreted must be derived, in the first place, from the blood.

The acid-base equilibrium system is a very complex one, employing, as it does, practically every system in the body.

The various factors for keeping the reaction of the blood constant are summarised as follows :—

The intrinsic properties of the blood.
The renal factor.
Metabolic functions.
Respiration.

The Intrinsic Properties of the Blood. The addition of acids or alkalis, except in very large quantities, to blood does not alter its reaction. This property is known as buffering, and is due to the presence of buffer substances. In blood the most important buffers are the proteins and salts, such as the phosphates Na_2HPO_4 and NaH_2PO_4, all of which are capable of adding on acids or alkalis with the formation of compounds which do not give free H or OH ions in solution. The free H ions are soaked up, and are consequently unable to influence the reaction. Sodium bicarbonate acts similarly in the blood, a neutral salt being formed by the action of an acid, with the liberation of the weak acid H_2CO_3. This is readily excreted in the form of H_2O and CO_2.

These buffer substances constitute the true alkali reserve. It is usual to estimate alkali reserve in the form of sodium bicarbonate, since it is rightly assumed that any persistent increase in the hydrogen ions would attack this first, and a measure of the blood bicarbonate would indicate the extent of the acid condition, a low figure pointing to a serious state.

A depletion of the alkali reserve, such as described above, is termed an *acidosis*, whilst an increase is known as an *alkalosis*. These terms refer to the quantity of available alkali present in the blood, and do not refer to the hydrogen ion concentration of the blood. It must be clearly understood that a patient may be in an extreme condition of acidosis with a normal hydrogen ion concentration of the blood. Depletion in the alkali reserve precedes a rise in the hydrogen ion concentration, and, hence, is a valuable indication for treatment.

The Van Slyke method for the estimation of alkali reserve is described at the end of this section.

The Renal Factor. The remarkable power of the kidney to secrete urine of widely varying reaction from the blood of constant hydrogen ion concentration has always attracted attention.

Bence-Jones (22) was the first to recognise the significance of this phenomenon, describing the alkaline urinary tide following a meal. This he ascribed to the removal of HCl from the blood during gastric secretion, thus tending to leave the blood in a more alkaline condition. The kidney then excretes alkaline urine in order to compensate the blood.

Metabolic Functions. In general, the metabolic processes incline towards the production of acid. Thus sulphur is oxidised to sulphuric acid, and lactic and butyric acids are produced in the course of fat metabolism, but in one particular process alkali is formed, and is used to neutralise the acids. It will be remembered that proteins, after being digested, are absorbed into the portal blood stream in the form of amino-acids. On reaching the liver these acids are deaminated, resulting in the setting free of an oxyacid and ammonia. Under normal circumstances the greater part of the ammonia is converted into urea.

If, however, stronger acids are present, the ammonia combines with them to form ammonium salts, thus constituting another factor in the neutrality regulating mechanism. A condition of acidosis will therefore raise the ammonia and decrease the urea content of the urine. This can best be expressed by working out the so-called ammonia co-efficient, *i.e.*, $\frac{\text{ammonia nitrogen}}{\text{total nitrogen}} \times 100$. Normally this is about 5%, and may be increased up to 10 to 18% in extreme conditions of acidosis. A rise in the ammonia co-efficient can be demonstrated very easily after starvation, either natural or due to vomiting.

Respiration. One of the methods of absorbing acid is by means of sodium bicarbonate. As has been stated already, the action of acid is to produce a neutral salt and CO_2, or rather H_2CO_3. Carbon dioxide stimulates the respiratory centre, with the result that the respiratory movements become much deeper, and consequently the ventilation is more efficient, and CO_2 is washed out of the blood. Any tendency to changes in the H ion concentration of the blood acts as a respiratory stimulus, producing exactly the same effects as CO_2.

A fall in the alveolar CO_2 tension indicates that the ventilation is increased, with the result that CO_2 is being washed out of the blood. This means a state of acidosis, whilst an increase indicates

alkalosis. The changes occurring after a meal in the alveolar CO_2 tension, as observed by one of us (E. C. D.) (23), support this theory. Normally the alveolar CO_2 tension varies from 35 to 45 mm., or, as a percentage, from 4 to 6·2. In acidosis the figures are much lower, 20 mm., or 1 to 2%.

Until recently, it was considered that when carbohydrate metabolism is deranged, as in diabetes mellitus, the fat metabolism is also affected.

It was thought that normally the complex long chain fatty acids were broken down two carbons at a time, until some simple substance, such as pyruvic acid resulted, which was then oxidised to CO_2 and water. In diabetes, however, the breaking-down process was considered to stop at the 4-carbon atom stage, thus leaving butyric acid. The body then tried to oxidise it away in the following series of reactions :—

$CH_3CH_2CH_2COOH$	Butyric acid.
$CH_3CHOHCH_2COOH$	β-oxy-butyric acid.
CH_3COCH_2COOH	Aceto-acetic acid.
$CH_3COCH_3 + CO_2$	Acetone.

The presence of these bodies in sufficient concentration produces the clinical condition known as diabetic coma.

β-oxy-butyric and aceto-acetic acids are substances which ionise, and consequently are capable of producing acidosis. There is evidence of acidosis in coma, since the alveolar CO_2 tension and the alkali reserve are lowered, and the question arises as to whether the condition is due entirely to acidosis, or whether, as some observers think, there is a specific pharmacological action of acetone bodies.

Some authorities hold the former view, and prefer to call the condition in diabetes *acidosis*, whilst others lean to the specific action theory and refer to the condition as *ketosis*. It has been stated that coma can be produced by the administration of neutral salts of aceto-acetic acid. This effect is said to be due to the presence of an enolic group $\begin{pmatrix} \text{COH} \\ \| \\ \text{CH} \end{pmatrix}$ (Hurtley and Trevan) (24).

Against this is the fact that workers have imbibed large quantities

of acetone bodies without any effect, whilst all observers estimate the alkali reserve with a view to ascertaining the onset of coma.

The above conception of the ætiology of diabetic acidosis is not in accord with recent experimental findings. Thus Mirsky et al. (25) experimenting with the heart-lung preparation, and using varying amounts of dextrose and acetone bodies in the perfusing blood, found no correlation between the utilisation of carbohydrate and the utilisation of acetone bodies, since the ketogenic-antiketogenic ratios varied from zero to infinity. In a review of the subject, Mirsky (26) stresses that sugar has no influence on the oxidation of acetone bodies, which are considered to originate in the liver and to be perfectly normal end products of fat metabolism. It is postulated that the production of acetone bodies is arrested by the presence of an adequate store of glycogen in the liver. Since glycogen is broken down at an excessive rate in diabetes, the stores of this substance in the liver may be rapidly depleted. If this occurs, the stage is set for an increase in fatty acid oxidation and an accelerated rate of formation of acetone bodies. Ketosis then results because the acetone bodies, which are not oxidised in the liver, are released into the blood stream at a rate which exceeds that at which they can be utilised by the muscles. Insulin is able to alleviate this condition because of its action in inhibiting glycogenolysis in the liver. In theory, by virtue of the tendency to promote the formation of glycogen in the liver, an excessive intake of carbohydrate should also tend to inhibit the development of ketosis in diabetes. In this connection, Mirsky et al. (27) found that a high carbohydrate intake produced a rise in blood sugar which was frequently associated with an inhibition of ketogenesis, but that the adoption of this procedure as a therapeutic measure was contra-indicated because of the possible dangers to the patient.

A series of cases of diabetic coma has been investigated by Dodds and Robertson (28). The alkali reserve, blood sugar, volatile and non-volatile acetone bodies were estimated in each patient at frequent intervals. It was shown that the degree of coma bore no relationship to the concentration of the volatile acetone bodies, and that in several of the cases in which complete recovery occurred the volatile acetone body content rose steadily.

The alkali reserve, however, was always low, and rose as the patient improved.

Methods of Investigating Acidosis and Ketosis

The practical importance of qualitative urinary tests for acetone bodies has already been emphasised, and it is only with the more elaborate procedure that we are concerned here. The estimation of the alkali reserve is usually performed on the patient in whom acidosis is suspected.

Van Slyke's Method for the Estimation of Alkali Reserve (29)

In this method the alkali reserve is estimated in the form of sodium bicarbonate. It consists in adding acid to a measured quantity of serum or plasma, and extracting the CO_2 so produced by reducing the pressure. The fluid is trapped by a very ingenious device, and the CO_2 is measured. The alkali reserve is calculated from this volume by reference to a table.

The actual CO_2 content of the blood, or else its CO_2 combining power, can be determined.

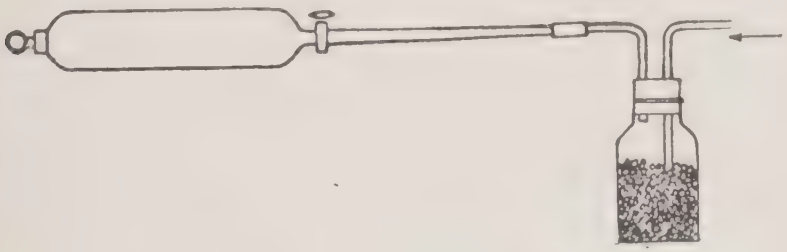

FIG. 1. Apparatus for Saturating Plasma with CO_2 (Van Slyke). The bottle contains glass beads.

Solutions. Phenolphthalein in alcohol (1%).

Caprylic alcohol.

5% sulphuric acid.

1% ammonia (carbonate free).

Ten ml. of blood are introduced into an oxalated centrifuge tube. The corpuscles are then centrifuged down, and as much plasma as possible is pipetted off into a separating funnel. This is placed on its side, and is filled with the operator's alveolar air. It is advisable to pass the breath through some form of con-

densation bottle first (see Fig. 1). The funnel is then rotated around its long axis in such a manner that the plasma is spread over the sides in the form of a thin film. Two minutes of this treatment are sufficient to saturate the plasma with CO_2. Alternatively, a mixture of 5% CO_2 in air from a cylinder may be bubbled through the plasma for three minutes, frothing being prevented by the addition of a few drops of caprylic alcohol. Whilst this is proceeding, the analysis apparatus is prepared. A line drawing of this appears on this page (Fig. 2), which is sufficiently clear without further explanation. The efficiency of the taps is tested by turning them off, and lowering the mercury reservoir, until a Torricellian vacuum is produced. If the taps are efficient, a sharp click will be heard on raising the reservoir. The taps F and E are set so that the reservoir B and trap D communicate with the main chamber A. The mercury reservoir is raised to position I when the mercury will just fill the capillary stem of B. The cup B is washed out with the ammonia solution, containing a drop of phenolphthalein solution. The washing is continued until the solution remains red, when all is pipetted off except a few drops. One ml. of plasma is then run into the cup from an Ostwald pipette, the point of which is kept under the surface of the ammonia solution. The tap E is then closed, and the mercury reservoir is lowered to position II. The tap E is then cautiously opened, and the liquid contained in the cup is run into the main reservoir until the upper surface of the liquid reaches the top

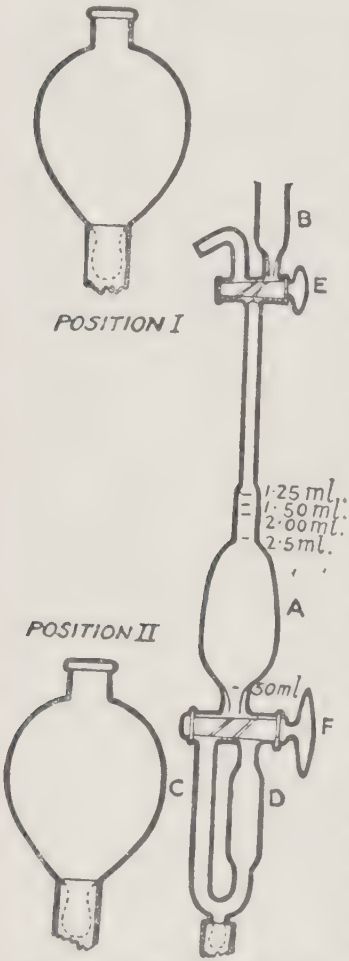

FIG. 2. Van Slyke's Apparatus for the Estimation of Alkali Reserve.

The reservoir is connected to the main apparatus by means of a rubber tube, the ends of which are shown cut across.

of the capillary stem, when the tap is shut. The cup is then washed with two lots of 0·5 ml. distilled water, each washing being run into the main chamber as before. A small drop of caprylic alcohol is then sucked into the chamber, followed by 0·5 ml. of the sulphuric acid solution. The tap E is then closed. The greatest care must be taken to avoid the introduction

Table I.

Barometer.	Barometer. 760	Barometer.	Barometer. 760
732	0·961	756	0·995
734	0·966	758	0·997
736	0·968	760	1·000
738	0·971	762	1·003
740	0·974	764	1·006
742	0·976	766	1·008
744	0·979	768	1·011
746	0·981	770	1·013
748	0·984	772	1·016
750	0·987	774	1·018
752	0·989	776	1·021
754	0·992	778	1·024

(Van Slyke.)

of air into the apparatus. The reservoir is now lowered still further, thus producing a Torricellian vacuum in the apparatus and causing CO_2 to be liberated. The mercury level inside the apparatus will now have fallen to the 50 ml. mark, and the tap F is closed. The apparatus is removed from the stand, and is shaken, in order to ensure thorough liberation of gas. After replacing in the stand, the tap F is opened, and the solution is allowed to run into the trap D. When the upper surface of the liquid reaches the top of the capillary in the tap, it is rapidly reversed and the mercury reservoir raised. The mercury will then run into the main chamber *viâ* the tube C, leaving the liquid trapped in D. The height of the reservoir is so arranged that the levels inside and outside the apparatus are the same, when the volume of the gas is read off.

Table II.

Table for Calculation of CO_2—Combining Power for Plasma.

Observed Vol. Gas × $\frac{B}{760}$	ml. of CO_2 reduced to 0° 760 mm., bound as Bicarbonate by 100 ml. of Plasma.				Observed Vol. Gas × $\frac{B}{760}$	ml. of CO_2 reduced to 0° 760 mm., bound as Bicarbonate by 100 ml. of Plasma.			
	15	20	25	30		15	20	25	30
0·20	9·1	9·9	10·7	11·8	0·60	47·7	48·1	48·5	48·6
1	10·1	10·9	11·7	12·6	1	48·7	49·0	49·4	49·5
2	11·0	11·8	12·6	13·5	2	49·7	50·0	50·4	50·4
3	12·0	12·8	13·6	14·3	3	50·7	51·0	51·3	51·4
4	13·0	13·7	14·5	15·2	4	51·6	51·9	52·2	52·3
5	13·9	14·7	15·5	16·1	5	52·6	52·8	53·2	53·2
6	14·9	15·7	16·4	17·0	6	53·6	53·8	54·1	54·1
7	15·9	16·6	17·4	18·0	7	54·5	54·8	55·1	55·1
8	16·8	17·6	18·3	18·9	8	55·5	55·7	56·0	56·0
9	17·8	18·5	19·2	19·8	9	56·5	56·7	57·0	56·9
0·30	18·8	19·5	20·2	20·8	0·70	57·4	57·6	57·9	57·9
1	19·7	20·4	21·1	21·7	1	58·4	58·6	58·9	58·8
2	20·7	21·4	22·1	22·6	2	59·4	59·5	59·8	59·7
3	21·7	22·3	23·0	23·5	3	60·3	60·5	60·7	60·6
4	22·6	23·3	24·0	24·5	4	61·3	61·4	61·7	61·6
5	23·6	24·2	24·9	25·4	5	62·3	62·4	62·6	62·5
6	24·6	25·2	25·8	26·3	6	63·2	63·3	63·6	63·4
7	25·5	26·2	26·8	27·3	7	64·2	64·3	64·5	64·3
8	26·5	27·1	27·7	28·2	8	65·2	65·3	65·5	65·3
9	27·5	28·1	28·7	29·1	9	66·1	66·2	66·4	66·2
0·40	28·4	29·0	29·6	30·0	0·80	67·1	67·2	67·3	67·1
1	29·4	30·0	30·5	31·0	1	68·1	68·1	68·3	68·0
2	30·3	30·9	31·5	31·9	2	69·0	69·1	69·2	69·0
3	31·3	31·9	32·4	32·8	3	70·0	70·0	70·2	69·9
4	32·3	32·8	33·4	33·8	4	71·0	71·0	71·1	70·8
5	33·2	33·8	34·3	34·7	5	71·9	72·0	72·1	71·8
6	34·2	34·7	35·3	35·6	6	72·9	72·9	73·0	72·7
7	35·2	35·7	36·2	36·5	7	73·9	73·9	74·0	73·6
8	36·1	36·6	37·2	37·4	8	74·8	74·8	74·9	74·5
9	37·1	37·6	38·1	38·4	9	75·8	75·8	75·8	75·4
0·50	38·1	38·5	39·0	39·3	0·90	76·8	76·7	76·8	76·4
1	39·1	39·5	40·0	40·3	1	77·8	77·7	77·7	77·3
2	40·0	40·4	40·9	41·2	2	78·7	78·6	78·7	78·2
3	41·0	41·4	41·9	42·1	3	79·7	79·6	79·6	79·2
4	42·0	42·4	42·8	43·0	4	80·7	80·5	80·6	80·1
5	42·9	43·3	43·8	43·9	5	81·6	81·5	81·5	81·0
6	43·9	44·3	44·7	44·9	6	82·6	82·5	82·4	82·0
7	44·9	45·3	45·7	45·8	7	83·6	83·4	83·4	82·9
8	45·8	46·2	46·6	46·7	8	84·5	84·4	84·3	83·8
9	46·8	47·1	47·5	47·6	9	85·5	85·3	85·2	84·8
0·60	47·7	48·1	48·5	48·6	1·00	86·5	86·2	86·2	85·7

(Van Slyke.)

Despite the ingenious method of trapping, a minute quantity of water will collect above the mercury, but if the reading is made at once no correction need be applied for the amount of CO_2 absorbed by this minute volume. The volume of gas is then reduced to standard pressure by multiplying by the factor obtained from Table I given on p. 99, taking the figure corresponding with the barometer reading for the day. By means of Table II. the temperature factor is allowed for, and by means of the right-hand columns the bicarbonate equivalent can be determined.

	CO_2 capacity, ml. per 100 ml.
Thirty normal students . . .	53 to 77
Acidosis, mild	44
Acidosis, severe. in terminal stages of chronic interstitial nephritis . .	34
Acidosis, severe, after anæsthesia . .	32
Fatal diabetic coma	21
Diabetes without coma . . .	34

If a large number of determinations have to be made daily, it is advisable to install special apparatus. The saturation of plasma with CO_2 is effected by passing a mixture of 5·5% CO_2 in air from a cylinder through the plasma for five minutes after the addition of caprylic alcohol. If a long glass tube, with a number of side tubes at right angles, is employed, a number of sera can be saturated at once. This method standardises the saturation, and eliminates the individual factor of the alveolar air, besides saving the operator much fatigue. The Van Slyke apparatus should also be incorporated on a vertical board, hinged to the base-board of the apparatus. This is connected to a wheel by an eccentrically placed connecting rod, so that on rotation of the wheel by a motor, mechanical shaking of the apparatus occurs. This should be done for one minute. By this means every part of the estimation is standardised and consistent results are obtained.

The normal limits of CO_2 capacity were found by Van Slyke to be from 53 to 77 ml. per 100 ml. of plasma.

The table above gives a rough idea of the findings in various types of cases.

The Treatment of Diabetes Mellitus

The starvation treatment of diabetes undoubtedly constituted a great therapeutic advance. It was introduced by Allen (30) in 1913, as the result of experiments on partially depancreatised dogs, and was followed by a considerable improvement in the prognosis of the disease. Guelpa (31), in 1910, had advocated periodical days of starvation combined with purgation in the treatment of diabetes. Graham (32), in 1917, also evolved a method of treatment by starvation somewhat similar to that of Allen.

The isolation of insulin by Banting and Best (1) in 1922, constituted a landmark, although it must be recognised that insulin is in no sense a cure, and in every instance its use must be combined with considerable dietetic restriction. Hagedorn (33) in 1935, introduced protamine insulin with a more delayed action, which was shortly followed by the production of protamine zinc insulin (see p. 118) and later by modifications such as histone zinc insulin, globin zinc insulin, and clear (soluble) zinc insulin. These have been investigated by Bailey and Marble (34), and globin insulin is now available for general use.

Since the introduction of the different varieties of insulin, there is no unanimity as to the best procedure in treating a case of diabetes. We shall therefore describe the methods which in our experience have proved successful.

Dietetic Treatment

It is necessary to know what constitutes an adequate dietary. The *basal requirement diet* varies with the weight and age of the patient. For practical purposes it may be reckoned that an adult requires 12 to 15 Cals. for every pound of body weight, *i.e.* :—

8 st.	. .	1,680 Cals. at 15 Cals. per lb.
9 st.	. .	1,890 Cals. ,, ,,
10 st.	. .	2,100 Cals. ,, ,,
11 st.	. .	2,310 Cals. ,, ,,
12 st.	. .	2,520 Cals. ,, ,,

Women require 10% less than adult men, children require proportionately more. These figures are the minimum, and do not allow for anything more than the lightest muscular activity.

In order that muscular work may be performed and weight maintained, additional calories will be required to the extent of 10% or 20%.

In building up a diet for a diabetic patient it is essential to know the constitution of the articles of food used as regards carbohydrate, protein and fat. This is usually expressed as grams per ounce of foodstuffs.

The caloric value of each article in the diet can then be easily calculated, as 1 gram of carbohydrate or protein is equivalent approximately to 4 Cals., and 1 gram of fat to 9 Cals. One-half to $\frac{3}{4}$ g. of protein per pound of body weight is required.

McCance and Shipp (35) have reported on the chemistry of flesh foods and their losses on cooking ; and McCance, Widdowson and Shackleton (36) have determined the nutritive value of fruits, vegetables and nuts. These analyses replace the figures obtained by previous investigations. We have classified the fruits and vegetables in six groups in accordance with these analyses. The nuts are grouped separately.

Carbohydrate			Carbohydrate
Group A = 0– 5%	. 1 oz.	containing	0 – 1·5 grams.
Group B = 5–10%	. 1 oz.	,,	1·5– 3 ,,
Group C = 10–15%	. 1 oz.	,,	3 – 4·5 ,,
Group D = 15–20%	. 1 oz.	,,	4·5– 6 ,,
Group E = 20–30%	. 1 oz.	,,	6 – 9 ,,
Group F = 50–65%	. 1 oz.	,,	15 –19·5 ,,

The articles are arranged alphabetically for purposes of reference,

Group A includes :—

VEGETABLES : Artichoke, Globe (boiled), 2·7% ; artichoke, Jerusalem (boiled), 3·2% ; asparagus tips (boiled), 1·1% ; beans, French (boiled), 1·1% ; beans, runner (boiled), 0·9% ; broccoli tops (boiled), 0·4% ; Brussels sprouts (boiled), 1·7% ; cabbage, savoy (boiled), 1·1% ; cabbage, spring (boiled), 0·8% ; cabbage, winter (boiled), 1·3% ; carrots, old (boiled), 4·3% ; carrots, young (boiled) 4·5% ; cauliflower (boiled), 1·2% ; celery (raw), 1·3% ; celery (boiled), 0·7% ; chicory (raw), 1·5% ; cucumber (raw), 1·8% ; egg plant (raw), 3·1% ; endive (raw), 1% ; leeks (boiled), 4·6% ; lettuce (raw), 1·8% ; marrow (boiled), 1·4% ; mustard and cress (raw), 0·9% ; mushrooms (raw or fried), 0% ; onions (boiled), 2·7% ; pumpkin (raw), 3·4% ; radishes (raw), 2·8% ; salsify (boiled), 2·8% ; seakale (boiled), 0·6% ; spinach (boiled), 1·4% ; spring greens (boiled), 0·9% ; swedes (boiled), 3·8% ; turnips (boiled), 2·3% ; turnip tops (boiled), 0·1% ; watercress (raw), 0·7%.

FRUITS : Apples (stewed), 4·4% ; Avocado pear (raw), 2·5% ; blackberries (stewed), 3·2% ; cherries (stewed), 4·1% ; cranberries (stewed), 1% ; currants, black (stewed), 4·6% ; currants, red (raw) 4·4% ; currants, red (stewed), 3·2% ; gooseberries (stewed), 1·7% ; lemon juice, 1·6% ; lemons, whole, 3·2% ; loganberries (raw), 3·4% ; melon, yellow, 5% ; olives, 0% ; raspberries, (stewed) 3·8% ; rhubarb (stewed), 0·7% ; tomatoes (fried), 3·3% ; tomatoes (raw), 2·8%.

Group B includes :—

VEGETABLES : Beans, broad (boiled), 7·1% ; beetroot (boiled), 9·9% ; onions, spring (raw), 8·5% ; peas, fresh (boiled), 7·7%.

FRUITS : Apricots (raw), 6·7% ; blackberries (raw), 6·4% ; currants, black (raw), 6·6% ; currants, white (raw), 5·6% ; damsons (raw), 9·6% ; damsons (stewed), 6·6% ; figs, green (raw), 9·5% ; gooseberries, ripe (raw), 9·2% ; grapefruit, 5·3% ; greengages (stewed), 7·6% ; melon, Cantaloupe, 5·3% ; mulberries (raw), 8·1% ; oranges, 8·5% ; orange juice, 9·4% ; passion fruit (raw), 6·2% ; peaches (raw), 9·1% ; pears (stewed), 6·5% ; plums, Victoria dessert (raw), 9·6% ; plums (stewed), 6·1% ; quinces (raw), 6·3% ; raspberries (raw), 5·6% ; strawberries (raw), 6·2% ; tangerines, 8%.

Group C includes :—

VEGETABLES : Horseradish (raw), 11% ; parsnips (boiled), 13·5%.

FRUITS : Apples, Empire eating (raw), 12·2% ; apples, English eating (raw), 11·7% ; cherries, eating (raw), 11·9% ; greengages (raw), 11·8% ; medlars (raw), 10·6% ; nectarines (raw), 12·4% ; pears, Empire eating (raw), 10·8% ; pears, English eating (raw), 10·4% ; pineapple (raw), 11·6% ; pomegranate juice, 11·6%.

Group D includes :—

VEGETABLES : Beans, baked (tinned), 17·3% ; beans, butter (boiled), 17·1% ; beans, haricot (boiled), 16·6% ; lentils (boiled), 18·3% ; peas, dried (boiled), 19·1% ; peas, tinned, 16·5% ; potatoes, old (boiled), 19·7% ; potatoes, new (boiled), 18·3%.

FRUITS : Apricots, dried (stewed), 18% ; banana (raw), 19·2% ; custard apple (raw), 18·1% ; grapes, black, 15·5% ; grapes, white, 16·1% ; peaches, dried (stewed), 18% ; prunes, dried (stewed), 15·5%.

Group E includes :—

VEGETABLES : Peas, split (soaked and boiled), 21·9% ; potatoes, sweet (boiled), 20·1%.

FRUITS : Figs, dried (stewed), 30%.

Group F includes :—

DRIED FRUITS : Currants, 63·1% ; dates, 63·9% ; figs, 52·9% ; raisins, 64·4% ; sultanas, 64·7%.

All fruits are stewed without the addition of sugar. Saccharin may be added as desired.

The fat content in these groups is so small as to be negligible, with the exception of Avocado pear, 8% fat; fried mushrooms, 6·7% fat; olives, 11% fat; fried tomatoes, 5·9% fat. The protein content in all these groups is so small that it can be neglected.

The composition of certain articles of food which are frequently used, and the groups into which they may be arranged are given below :—

Average Carbohydrate Content of Vegetables or Fruits

		Grams			
		Carbohydrate	Protein	Fat	Calories
Group A . . .	1 oz.	0·75	0	0	3
Group B . . .	1 oz.	2·25	0	0	9
Group C . . .	1 oz.	3·75	0	0	15
Group D . . .	1 oz.	5·25	0	0	21
Group E . . .	1 oz.	7	0	0	28
Group F . . .	1 oz.	17·25	0	0	69

Interchangeability of Vegetable or Fruit Groups. It will be noted that the following approximate relationship exists between the mean carbohydrate content of these groups :—

Group A 12 oz. ≡ Group B 4 oz. ≡ Group C $2\frac{1}{2}$ oz. ≡ Group D $1\frac{3}{4}$ oz. ≡ Group E $1\frac{1}{3}$ oz. ≡ Group F $\frac{1}{2}$ oz.

Nuts :		Grams			
		Carbo-hydrate	Protein	Fat	Calories
Chestnut . . .	1 oz.	10·9	0·7	0·8	54
Coconut . . .	1 oz.	1·1	1·1	10·8	106
Walnut . . .	1 oz.	1·5	3·7	15·4	159
Almonds . . .	1 oz.	1·3	6·1	16	174
Brazil . . .	1 oz.	1·2	4·1	18·4	187
Barcelona . . .	1 oz.	1·6	3·9	19·2	195

For the purposes of alternatives in the diet, chestnuts may be taken as a Group C vegetable.

Farinaceous Foods :		Grams			
		Carbo-hydrate	Protein	Fat	Calories
Bread (white) . . .	1 oz.	14·3	2	0·4	69
Bread (brown) . . .	1 oz.	13	1·4	0·4	61
Toast (white) . . .	1 oz.	17·2	2·3	0·3	81
Toast (fairy) . . .	1 oz.	21	3	0·6	103
Energen bread (1 roll) .		2	1·5	0·25	16
Ryvita (3 sections) . .	1 oz.	21·2	3·3	0·4	105
Vitaweat (3 sections) .	1 oz.	21·2	3·3	2·2	121
Rice (uncooked) . .	1 oz.	22·7	1·6	0·1	98
Oatmeal (dry) . .	1 oz.	18	4·6	2	108

Dairy Produce :		Grams			
		Carbohydrate	Protein	Fat	Calories
Egg (1)	2 oz.	0	7·6	4·8	74
Milk	1 oz.	1·5	1	1	19
Butter	1 oz.	0	0	25	225
Cream (20%)	1 oz.	1	1	6	62
Cheese :					
Camembert	1 oz.	0	6·3	6·5	84
Dutch	1 oz.	0	9·2	5·3	84
Wensleydale	1 oz.	0	10	6	94
Gorgonzola	1 oz.	0·5	7·8	8	105
Gruyère	1 oz.	0	9·4	8·5	114
Cheddar	1 oz.	0·9	10	8	116
Cheshire	1 oz.	1·3	8·8	9·2	123
St. Ivel	1 oz.	0	7·1	10·5	123
Roquefort	1 oz.	0·5	10·4	9·4	128
Stilton	1 oz.	0	7·2	11·7	134
CHEESE GROUP A :					
Camembert, Dutch, Wensleydale, Gorgonzola, Gruyère, Cheddar :					
Average Content	1 oz.	0·2	8·8	7	99
CHEESE GROUP B :					
Cheshire, St. Ivel, Roquefort, Stilton :					
Average Content	1 oz.	0·4	8·4	10·2	127

Interchangeability of Cheese Groups. Group A, 1 oz. ≡ Group B, $\frac{3}{4}$ oz.

Preserved Meats :		Grams			
		Carbohydrate	Protein	Fat	Calories
Bacon (back, fried)	1 oz.	0	7·4	16	174
Bacon (streaky, fried)	1 oz.	0	7·2	13·8	153
Ham (lean, boiled)	1 oz.	0	6·9	1	64
Tongue (ox, canned)	1 oz.	0	5·8	7	86
Fresh Meat :					
Rabbit (stewed)	1 oz.	0	8	2·3	53
Hare (stewed or roast)	1 oz.	0	8·8	2·4	57
Veal (cutlet, fried)	1 oz.	0	9·1	2·4	58
Beef (roast)	1 oz.	0	8	3·7	65
Veal (fillet, roast)	1 oz.	0	9·2	3·5	68
Mutton (boiled)	1 oz.	0	7·7	5	76
Mutton (roast)	1 oz.	0	7·5	6·1	85
Pork (leg, roast)	1 oz.	0	7·4	7	93
Pork (loin, roast)	1 oz.	0	7·8	10·7	128

		Grams			
Poultry and Game :		Carbo-hydrate	Protein	Fat	Calories
Grouse (roast)	1 oz.	0	9	1·6	50
Chicken (roast)	1 oz.	0	8·9	2·2	55
Turkey (roast)	1 oz.	0	9	2·3	57
Chicken (boiled)	1 oz.	0	7·9	3·1	60
Guinea fowl (roast)	1 oz.	0	9·8	2·5	62
Partridge (roast)	1 oz.	0	10·6	2·2	62
Pheasant (roast)	1 oz.	0	9·2	2·8	62
Pigeon (roast)	1 oz.	0	8	4	68
Duck (roast)	1 oz.	0	6·8	7·1	91
Goose (roast)	1 oz.	0	8·4	6·7	94
Internal Organs :					
Brain (calf, sheep, boiled)	1 oz.	0	3·5	1·9	31
Kidney (stewed)	1 oz.	0	7·7	1·7	46
Sweetbread (stewed)	1 oz.	0	6·8	2·7	52
Kidney (fried)	1 oz.	0	8·4	2·7	58
Liver (calf, fried after rolling in flour)	1 oz.	0·7	8·7	4·4	77
Tongue (sheep, fresh, stewed)	1 oz.	0	5·5	7·2	87
Fresh Meat, Ham, Tongue, Poultry, Game and Internal Organs, Groups :					
GROUP A :					
Rabbit, hare, veal, beef, mutton (boiled), grouse, chicken, turkey, guinea-fowl, partridge, pheasant, pigeon, brains, kidneys, sweetbread, liver, ham. (Served as above.) *Average Content*	1 oz.	0	8·3	2·9	59
GROUP B :					
Mutton (roast), pork, tongue, duck, goose. (Served as above.) *Average Content*	1 oz.	0	7	7·4	95

Interchangeability of Meat Groups.

Group A 3 oz. ≡ Group B $1\frac{7}{8}$ oz.

Group A 2 oz. ≡ Group B $1\frac{1}{4}$ oz.

Fish :		Grams			Calories
		Carbo-hydrate	Protein	Fat	
Oysters	1 oz.	0	3	0·3	15
Cod (steamed)	1 oz.	0	5·4	0·3	24
Sole (steamed)	1 oz.	0	5·3	0·4	25
Lemon sole (steamed)	1 oz.	0	6	0·3	27
Plaice (steamed)	1 oz.	0	5·4	0·6	27
Whiting (steamed)	1 oz.	0	6	0·3	27
Haddock (steamed)	1 oz.	0	6·6	0·2	28
Turbot (steamed)	1 oz.	0	6·2	0·5	29
Haddock (smoked, steamed)	1 oz.	0	6·7	0·3	30
Hake (steamed)	1 oz.	0	5·5	1	31
Lobster (boiled)	1 oz.	0	6·4	1	35
Trout (river, steamed)	1 oz.	0	6·7	1	36
Crab (boiled)	1 oz.	0	5·8	1·6	38
Halibut (steamed)	1 oz.	0	6·8	1·2	38
Mullet (red or gray, steamed)	1 oz.	0	6·5	1·3	38
Salmon (tinned)	1 oz.	0	5·9	1·8	40
Herring (baked in vinegar)	1 oz.	0	5	3·9	55
Salmon (steamed)	1 oz.	0	5·7	3·9	58
Kippers (baked)	1 oz.	0	7	3·4	59
Sardines (tinned)	1 oz.	0	6·1	6·8	86
Fish Group A :					
Cod, sole, lemon sole, plaice, whiting, haddock, turbot. (Served as above.) *Average Content*	1 oz.	0	5·8	0·4	27
Fish Group B :					
Hake, lobster, trout, halibut, mullet, crab, tinned salmon. (Served as above.) *Average Content*	1 oz.	0	6·2	1·3	36
Fish Group C :					
Herring, salmon, kippers, sardines. (Served as above.) *Average Content*	1 oz.	0	5·9	4·5	64

Interchangeability of Fish Groups

Group A 3 oz. ≡ Group B $2\frac{1}{4}$ oz. ≡ Group C $1\frac{1}{4}$ oz.
Group A 2 oz. ≡ Group B $1\frac{1}{2}$ oz. ≡ Group C $\frac{5}{6}$ oz.

Miscellaneous :		Grams			
		Carbohydrate	Protein	Fat	Calories
Pickles (mixed, unsweetened)	1 oz.	1·2	0·2	0·1	25
Olive oil	1 oz.	0	0	30	270
Whisky (25% alcohol) .	1 oz.	—	—	—	50
Lump sugar (8 lumps, $\frac{5}{8}$-in. cube)	1 oz.	30	0	0	120

Attention should be paid to the following points in building up the diets. The weights of the foodstuffs in the diets (see p. 111) refer to the tables of the composition of foods given above. Thus the vegetables and fruits are weighed as served, and the meat and fish are weighed cooked as indicated in the tables. The chief carbohydrate-containing meals are given morning and evening, and this is especially important if soluble insulin is being administered twice daily.

We have classified the vegetables, fruits, cheese, fish and meat into groups from which equivalent quantities can be selected. This allows considerable variation to be made in the diets with the minimum of trouble, and the diets should always be made as attractive as possible for the diabetic patient. Thus with the vegetables and fruits, variations should be made in and between the groups. For example, instead of 6 oz. of one article in Group A, the patient may be given a variety of articles in the same group, such as a salad of tomatoes 2 oz., radishes $1\frac{1}{2}$ oz., lettuce 1 oz., cucumber 1 oz., and mustard and cress $\frac{1}{2}$ oz. The equivalent quantities of the higher carbohydrate-containing groups may also be used. Thus for Veg. A 6 oz., the following are examples of alternatives: Tomatoes 2 oz., lettuce 1 oz., cucumber 1 oz., and beetroot $\frac{2}{3}$ oz.; or again, lettuce 1 oz., spring onions $\frac{5}{6}$ oz., fresh peas (boiled) $\frac{5}{6}$ oz.; or stewed apple 3 oz., and fried or raw tomato 3 oz.: or orange 2 oz.; or raw apple $1\frac{1}{4}$ oz. Further, instead of Veg. A 12 oz., potato $1\frac{3}{4}$ oz., or banana $1\frac{3}{4}$ oz., or dried figs $1\frac{1}{3}$ oz., or dates $\frac{1}{2}$ oz. may be substituted. Due use should be made of the fruits in the daily menu. The patient's likes and dislikes as regards fish, meat, poultry and game should always be consulted. The patient should select what he fancies from the dietary, the amounts allowed being determined from the group figures. Thus for fish, $2\frac{1}{4}$ oz. of Group B or $1\frac{1}{4}$ oz. of Group C may be used instead of 3 oz. of Group A, and for meats

$1\frac{7}{8}$ oz. of Group B are equivalent to 3 oz. of Group A. If the patient wishes small meals the requisite calories can be supplied by using the concentrated foods, alternatively if he is hungry and desires larger meals the foods less rich in protein, fat and carbohydrate can be ordered. If the tables are used intelligently no diabetic patient will be given a monotonous diet or one which is more than he can eat or too small to satisfy his hunger. The division of the foodstuffs into the above groups, using the average carbohydrate, protein and fat values as the basis of the calculation of the food value of any substance included in a group, is theoretically permissible and practically satisfactory in the treatment of diabetes. This is so because even if the physician orders a diet with meticulous accuracy as regards the carbohydrate, protein and fat in each ingredient, there are many sources of error between the ordering of the diet and its metabolism by the patient. Thus the composition of the actual foods eaten by the patient may differ slightly from those upon which the laboratory analyses were made. A variable error in weighing, cooking and serving the diet will also occur, which may be as much as + or — 10% in the day's food. Further, individuals vary as regards the extent to which they absorb and metabolise the foodstuffs.

To make the diets more attractive the following substances are permissible : Salt, pepper, mustard, lemon juice, gelatine, rennet, vinegar, almond essence, cloves, parsley, sage, vanilla essence, mixed herbs and small quantities of anchovy sauce, Worcestershire sauce and Gentleman's relish. Saccharin in gr. $\frac{1}{3}$ tablets may be used for sweetening stewed fruits, etc. Olive oil may be substituted for a portion of the butter allowance to make salad dressing with vinegar and a little cream.

The rationing system which is enforced in Great Britain owing to the war involves some modification of the standard diets in diabetes. The diabetic is allowed three meat rations in exchange for his sugar ration and he is also given a treble fat ration. Vitaminised margarine can be used instead of butter.

The patient should be provided with folding portable scales with a swing balance, capable of weighing from $\frac{1}{8}$ oz. to 18 oz. A plate or thin piece of paper should be put on the scales and the requisite amount of food is then weighed. Fluid articles of diet should be measured in a graduated medicine glass. It is

generally found that after a time the patient becomes a good judge of the weight of different articles of food, and so it is not essential at all times for them to be weighed. Thus when dining out, or on short visits to a hotel, the amount of foodstuffs allowed can be fairly accurately gauged. This practice should not, however, be encouraged as a routine, as it favours slipshod habits which would, if permitted, sooner or later prove the patient's undoing.

Attention is again drawn to the following points as regards variations in the diets which follow :—

Vegetables and Fruits :

Group A 12 oz. are equivalent to Group B 4 oz.
Group A 12 oz. ,, ,, ,, Group C $2\frac{1}{2}$ oz.
Group A 12 oz. ,, ,, ,, Group D $1\frac{3}{4}$ oz.
Group A 12 oz. ,, ,, ,, Group E $1\frac{1}{3}$ oz.
Group A 12 oz. ,, ,, ,, Group F $\frac{1}{2}$ oz.

Farinaceous Foods :

Fairy toast, $\frac{2}{3}$ oz. may be substituted for Bread (white) 1 oz.
Vitaweat, $\frac{1}{3}$ section ,, ,, 1 Energen roll.
Ryvita, $\frac{1}{3}$ section ,, ,, 1 Energen roll.

Cheese :

Group A 1 oz. is equivalent to Group B $\frac{3}{4}$ oz.

Meat : This includes fresh meat, ham, tongue, poultry, game and internal organs.

Group A 3 oz. are equivalent to Group B $1\frac{7}{8}$ oz.
Group A 2 oz. ,, ,, ,, Group B $1\frac{1}{4}$ oz.

Fish :

Group A 3 oz. are equivalent to Group B $2\frac{1}{4}$ oz.
Group A 3 oz. ,, ,, ,, Group C $1\frac{1}{4}$ oz.
Group A 2 oz. ,, ,, ,, Group B $1\frac{1}{2}$ oz.
Group A 2 oz. ,, ,, ,, Group C $\frac{5}{6}$ oz.

Low Carbohydrate Diets

Diet 1. **Breakfast :** Veg. or Fruit Group A, 3 oz. ; Egg, 1 ; Tea. **Lunch :** Veg. or Fruit Group A, 2 oz. ; Bovril, 10 oz. ; Water. **Tea :** Veg. or Fruit Group A, 2 oz. ; Tea. **Dinner :**

Veg. or Fruit Group A, 3 oz.; Egg, 1; Bovril, 10 oz.; Water. **C.** 7 g., **P.** 15 g., **F.** 10 g. **Calories** = 178.

Diet 2. **Breakfast:** Veg. or Fruit Group A, 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Tea. **Lunch:** Veg. or Fruit Group A, 3 oz.; Meat Group A, 2 oz.; Butter, ¼ oz.; Energen bread, 1 roll; Bovril and Water, as desired. **Tea:** Veg. or Fruit Group A, 3 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Bovril and Water, as desired. **C.** 15 g., **P.** 36 g., **F.** 35 g. **Calories** = 519.

Diet 3. **Breakfast:** Veg. or Fruit Group A, 6 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Bacon (streaky), 1 oz.; Milk, 1 oz.; Tea. **Lunch:** Veg. or Fruit Group A, 6 oz.; Meat Group A, 2 oz.; Butter, ½ oz.; Energen bread, 1 roll; Cheese Group A, ½ oz.; Water. **Tea:** Veg. or Fruit Group A, 6 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 6 oz.; Egg, 1; Butter, ½ oz.; Energen bread, 1 roll; Fish Group A, 2 oz.; Meat Group A, 2 oz.; Water. **C.** 31 g., **P.** 77 g., **F.** 78 g. **Calories** = 1,134.

Diet 4. **Breakfast:** Veg. or Fruit Group A, 6 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Bacon (streaky), 1 oz.; Oatmeal (dry), ½ oz.; Milk, 2 oz.; Cream (20%), ½ oz.; Tea. **Lunch:** Veg. or Fruit Group A, 6 oz.; Meat Group A, 2 oz.; Butter, ½ oz.; Energen bread, 1 roll; Cheese Group A, 1 oz.; Water. **Tea:** Veg. or Fruit Group A, 6 oz.; Butter, ¼ oz.; Energen bread, 1 roll; Milk, 1 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 6 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Fish Group A, 2 oz.; Meat Group A, 2 oz.; Water. **C.** 50 g., **P.** 87 g., **F.** 94 g. **Calories** = 1,394.

Diet 5. **Breakfast:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Bacon (streaky), 2 oz.; Oatmeal (dry) ½ oz.; Milk, 2 oz.; Cream (20%), ½ oz.; Tea. **Lunch:** Veg. or Fruit Group A, 6 oz.; Meat Group A, 3 oz.; Butter, ½ oz.; Bread, ½ oz.; Cheese Group A, 1 oz.; Water. **Tea:** Veg. or Fruit Group A, 6 oz.; Butter, ¼ oz.; Energen bread, 1 roll; Milk, 1 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Fish Group A, 2 oz.; Meat Group A, 3 oz.; Cream (20%), ½ oz.; Water; Coffee. **C.** 65 g., **P.** 111 g., **F.** 117 g. **Calories** = 1,757.

Diet 6. **Breakfast:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Bacon (streaky), 2 oz.; Oatmeal

(dry), 1 oz.; Milk, 2 oz.; Cream (20%), ½ oz.; Tea. **Lunch:** Veg. or Fruit Group A, 6 oz.; Meat Group A, 3 oz.; Butter, ½ oz.; Bread, ½ oz.; Cheese Group A, 1 oz.; Water. **Tea:** Veg. or Fruit Group A, 6 oz.; Butter, ¼ oz.; Energen bread, 1 roll; Milk, 1 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Fish Group A, 3 oz.; Meat Group A, 3 oz.; Cream (20%), ½ oz.; Water; Coffee. **C.** 74 g., **P.** 119 g., **F.** 118 g. **Calories** = 1,834.

Diet 7. **Breakfast:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Bacon (streaky), 2 oz.; Oatmeal (dry), 1 oz.; Milk, 3 oz.; Cream (20%), 1 oz.; Tea. **Lunch:** Veg. or Fruit Group A, 6 oz.; Meat Group A, 3 oz.; Butter, ½ oz.; Bread, ½ oz.; Cheese Group A, 1½ oz.; Water. **Tea:** Veg. or Fruit Group A, 6 oz.; Butter, ¼ oz.; Bread, ½ oz.; Milk, 1 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ¾ oz.; Bread, 1 oz.; Fish Group A, 3 oz.; Meat Group A, 3 oz.; Cream (20%), ½ oz.; Water; Coffee. **C.** 89 g., **P.** 125 g., **F.** 132 g. **Calories** = 2,044.

Diet 8. **Breakfast:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ½ oz.; Bread, ½ oz.; Bacon (streaky), 2 oz.; Oatmeal (dry), 1 oz.; Milk, 3 oz.; Cream (20%), 1 oz.; Tea. **Lunch:** Veg. or Fruit Group A, 6 oz.; Meat Group A, 3 oz.; Potato, 1½ oz.; Butter, ½ oz.; Bread, ½ oz.; Cheese Group A, 1½ oz; Water. **Tea:** Veg. or Fruit Group A, 6 oz.; Butter, ¼ oz.; Bread, ½ oz.; Milk, 1 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ¾ oz.; Bread, 1 oz.; Fish Group A, 3 oz.; Meat Group A, 3 oz.; Potato, 1½ oz.; Cream (20%), ½ oz.; Water; Coffee. **C.** 106 g., **P.** 126 g., **F.** 132 g. **Calories** = 2,116.

Diet 9. **Breakfast:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ½ oz.; Bread, 1 oz.; Bacon (streaky), 2 oz.; Oatmeal (dry), 1 oz.; Milk, 3 oz.; Cream (20%), 1 oz.; Tea. **Lunch:** Veg. or Fruit Group A, 6 oz.; Meat Group A. 3 oz.; Potato, 1½ oz.; Butter, ¾ oz.; Bread, 1 oz.; Cheese Group A. 1½ oz.; Water. **Tea:** Veg. or Fruit Group A, 6 oz.; Butter, ½ oz.; Bread, 1 oz.; Milk, 1 oz.; Tea. **Dinner:** Veg. or Fruit Group A, 12 oz.; Egg, 1; Butter, ¾ oz.; Bread, 1½ oz.; Fish Group A, 3 oz.; Meat Group A, 3 oz.; Potato, 1½ oz.; Cream (20%) 1 oz.; Water; Coffee. **C.** 135 g., **P.** 131 g., **F.** 149 g. **Calories** = 2,405.

The Use of Low Carbohydrate Diets

Some authorities advise placing the patient at once on his full diet, and working out the insulin to balance it. Such a diet can contain a low or a high amount of carbohydrate.

Low carbohydrate diets, with a carbohydrate content of 100 to 140 g., have a very definite place in the treatment of diabetes. The method of preliminary starvation, followed by a series of graduated low carbohydrate diets, will often enable a patient to be stabilised on an adequate diet without the use of insulin. Further, in some cases the patient can be rendered sugar free without preliminary starvation and without insulin by the use of an intermediate low carbohydrate diet. He can then be worked up to an adequate diet with or without insulin according to his tolerance. High carbohydrate, low fat diets with insulin are usually necessary for children and for severe cases of diabetes in young adults.

Method. The patient is put to bed and given an ordinary standard diet of known calorie value, such as diet No. 4 (see p. 112). The urine is tested for sugar and acetone bodies and the sugar tolerance curve and alkali reserve are determined. In this way the diagnosis of diabetes will be established and the severity of the case ascertained. Insulin should be used at once in cases of diabetes with complications such as marked acidosis, tuberculosis, gangrene or neuritis. If there is acidosis ½ to 1 oz. of dextrose should be given with breakfast and dinner, half an hour after the insulin injections. When the acidosis disappears the dextrose can be gradually omitted and the diets increased as described later. In uncomplicated cases the routine procedure is as follows :—

If the urine is sugar free on diet No. 4, the patient should be allowed to get up and walk, at first in his room, and later out of doors when the diets are increased. Exercise tends to lower the blood sugar. The diet is increased every other day until the patient is taking a diet 20% in excess of his basal requirement (see p. 102). Providing the urine remains sugar free no insulin will be required. If the urine is not sugar free after remaining on diet No. 4 for three or four days, two courses are open : The patient may be starved and worked up through the lower diets,

or, keeping the patient on diet No. 4, insulin may be given to render the urine sugar free and the diets then increased as described above, the patient getting up in either case when he is taking diet No. 4. The advantage of starvation is that in some cases the patient may subsequently be able to take an adequate dietary without the use of insulin. As stated above starvation must not be attempted in severe cases in which there is marked acidosis, as the sudden deprivation of carbohydrate food may precipitate coma. Children also should never be starved. In the vast majority of cases, however, starvation can be undertaken with impunity, as the acidosis due to starvation is not toxic. During the starvation period, which in some cases may be prolonged for four or five days, the patient should drink four or five pints of fluid a day in the form of water, weak tea or coffee (without sugar or milk), meat extracts and clear meat soups. Small quantities of whisky or brandy may be given during this stage, if desired. Care should be taken to prevent constipation. The urine usually becomes sugar free in twenty-four to forty-eight hours, and as soon as it is sugar free diet No. 1 is given. Should any signs of coma appear the patient must be promptly treated with insulin and dextrose, as detailed on p. 132, when a ready response will usually be obtained. If the urine is not sugar free after the starvation period insulin will be required, and the patient should be given diet No. 1 and subsequently worked up through the diets.

The urine should be tested daily for sugar and acetone bodies before breakfast, lunch, tea and dinner. These observations should be recorded on a chart. When the patient has reached an adequate diet and is sugar free, a blood sugar reading should be made at 12 noon to make sure the blood sugar is within normal limits and that the renal threshold is not higher than normal. If insulin is required, 5 units of soluble insulin are injected half an hour before breakfast and this dose increased by 2 units at a time until the midday specimen of urine is sugar free. If the specimen of urine passed before breakfast contains sugar, 5 units of soluble insulin are also given before dinner and this dose increased by 2 units at a time until the early morning specimen of urine is also sugar free. A smaller dose of insulin is usually required in the evening, as the carbohydrate content of the evening meal is less than that of the morning one. An interval

of two or three days should elapse between each morning and evening increment of insulin, as in this way the danger of giving an overdose is reduced to a minimum. It is not advisable to give a single dose of more than 40 units of soluble insulin unless blood sugar estimations are being made, except as an emergency measure in diabetic coma. If glycosuria therefore persists when the insulin has been increased to 40 units in the morning and to 30 units in the evening, it will probably be necessary to give a third injection of insulin before lunch, or to use a protamine insulin as described later. In cases in which glycosuria persists despite the administration of insulin and in which a further increase of insulin results in malaise, lack of appetite or other hypoglycæmic symptoms (see p. 136), blood sugar readings may show that the renal leak point is low, sugar appearing in the urine when the blood sugar is lower than the normal leak point of 180 mg. per 100 ml. In such cases a certain degree of glycosuria is permissible and indeed requisite for the patient's well-being. Should hypoglycæmic symptoms occur at any stage of treatment the patient should eat one or two lumps of sugar.

After the patient is stabilised on an adequate dietary a search should be made for any possible focus of sepsis. The teeth and cranial sinuses should be X-rayed, and the tonsils and naso-pharynx investigated by a throat specialist. If there are any symptoms suggestive of chronic cholecystitis or appendicitis the appropriate X-ray examination should be carried out. A catheter specimen of urine should also be examined for organisms. It has been shown that sepsis is often associated with hyperglycæmia, and possibly it is causally related thereto. If an anæsthetic is required for the removal of a septic focus the necessary preparation should be carried out as described on p. 125.

The Use of High Carbohydrate Diets

Rabinowitch (37) was largely responsible for the successful introduction of a diabetic diet containing considerable amounts of carbohydrate, such as 200 to 300 g. It had long been realised that fats are a potential source of danger in diabetes, as, if insufficient glycogen is deposited in the liver, they lead to ketosis. In addition to the high carbohydrate content, there are two out-

standing features in the diabetic diets advocated by Rabinowitch ; both the total calorie value and the fat content are low. The patient is kept permanently slightly undernourished, so that he is about 5 lb. below his " proper " weight. It is also important that the fat content of the diet be small, usually about 50 g. a day. As Rabinowitch remarks, if this is not done and the patient is given a high carbohydrate diet, then he is receiving no treatment at all. Rabinowitch points out the danger of exceeding the proper fat allowance, and shows how this will cause the treatment to fail, as when a patient inadvertently took fat bacon instead of lean bacon for breakfast. We have carefully treated a series of severe cases of diabetes on the strict Rabinowitch *régime* and can vouch for its efficacy. Thus we have tried it on carefully worked out cases who have been in diabetic coma on low carbohydrate diets with insulin, and who have required very careful watching to maintain health. The patient's height is recorded and his " proper " weight determined according to the Rabinowitch table. The modern tendency is to increase the fat content of the diet to about 80 g., and also to give a larger calorie value than in the original Rabinowitch *régime*.

Proper Weight of Patient 5 Feet High

(Three pounds are to be added for every inch over 5 feet. If the patient is a woman, 5 lb. are to be subtracted from the total.)

Age 15–25	120 lb.
Age 26–30	125 ,,
Age 31–35	130 ,,
Over 35	135 ,,

The calories required are worked out on a basis of 15 cals. per lb., 5 lb. having been subtracted from the patient's estimated proper weight. High carbohydrate diets are of particular value in children, young adults, and when the disease is complicated by nephritis, peptic ulcer and pulmonary tuberculosis. The following is an example of a diet used for a severe case, the patient being a girl aged fourteen. The insulin dosage required in this case is protamine zinc insulin 56 units at night, and soluble insulin 10 to 15 units before breakfast.

7 a.m. Two biscuits ($\frac{1}{2}$ oz.). **Breakfast :** Apple, 2 oz. ; Egg, 1 ;

Bread, 1 oz.; Oatmeal (dry), 1 oz.; Jam, ½ oz.; Milk, 6 oz.; Sugar, 2½ level teaspoonfuls. **Mid-morning :** Bread, 1 oz. or 3 biscuits (¾ oz.). **Lunch :** Bovril, 10 oz.; Lean meat, 1½ oz.; Potato (cooked), 3 oz.; Butter, ⅓ oz.; Apple, 2 oz.; Veg. Group A, 3 oz.; Bread, 1 oz. **Tea :** Milk, 4 oz.; Bread, 2 oz.; Plain cake, 1 piece (2 oz.); Jam, ½ oz.; Banana, 1, or Bread, 1 oz. **Supper :** Fish, 1½ oz.; Potato (cooked), 3 oz.; Butter, ⅓ oz.; Apple, 2 oz.; Milk, 4 oz.; Veg. Group A, 3 oz.; Bread, 1 oz.; Bananas, 2, or Bread, 2 oz.

10 p.m. Milk, 4 oz.; Plain cake, 1 piece (2 oz.) or 3 biscuits (¾ oz.). **C.** 309 g., **P.** 81 g., **F.** 68 g. **Calories** = 2,172.

The Protamine Insulins

From time to time attempts have been made to lower the rate of absorption of insulin and so to prolong its action. Various substances were added to insulin, such as gum arabic, protein, fat, oil, tannic acid, lecithin, pituitrin and adrenaline; but all with unsatisfactory results.

In 1935 Hagedorn (33), in Copenhagen, produced protamine insulinate (protamine insulin, insulin retard) which had the desired prolonged effect. This was prepared by combining a solution of insulin hydrochloride with a protamine obtained from the sperm of a species of trout, the *salmo irideus*.

Scott and Fisher (38) in 1936 modified protamine insulin by adding zinc to it in the proportion of 1 mg. to 500 units. This preparation is known as protamine zinc insulin (protamine insulin (with zinc) suspension).

A study of blood sugar curves in the non-diabetic subject, after the injection of the varieties of insulin now commonly employed, indicates the rationale of their use in the treatment of diabetes. Thus Bennett and his co-workers (39) have shown that with ordinary or soluble insulin the blood sugar is lowered in from twenty to forty minutes, the maximum effect being produced in three hours. With protamine insulin the corresponding figures are one to three hours for the initial effect and six to eight hours for the maximum action, and with protamine zinc insulin the blood sugar begins to fall in nine to eleven hours, the greatest effect being obtained in fifteen to twenty hours. Mosenthal (40) states that protamine zinc insulin often lowers the blood sugar for longer

than twenty-four hours. Aitken (41) has, however, shown that in diabetes protamine zinc insulin produces an effect earlier. He determined the urinary sugar excretion of diabetic patients receiving constant diets, and found that protamine zinc insulin exerts a large and possibly maximal insulin effect within three to six hours of injection, and the total duration of the effect of doses between 15 and 100 units is fifteen to sixty hours.

Methods of Administration

Protamine zinc insulin is put up in single, double or quadruple strengths (20, 40 or 80 units in 1 ml.). It is considered to remain potent for periods up to six months, but in order to ensure sterility the suspension should be used within fourteen days of the withdrawal of the first dose.

Indications for Protamine Zinc Insulin

This is chiefly of value when only one injection of insulin is desired by patients requiring two or more daily injections of soluble insulin. There is considerable difference of opinion whether protamine zinc insulin should be injected in the morning or the evening. Mosenthal and Mark (42) say that as it acts for more than twenty-four hours the time of injection would seem to be immaterial, but patients usually find an injection before breakfast most convenient. Some patients, however, can only be controlled satisfactorily by an evening injection. If after a few days' trial with a morning injection of protamine zinc insulin it is not possible to control the rise of blood sugar after breakfast an injection of soluble insulin will also be required. Joslin (43) advises separate injections, using the left arm or leg for the soluble insulin and the right arm or leg for the protamine insulin. If the same syringe is used the soluble insulin should be injected first, otherwise the traces of protamine zinc insulin remaining in the syringe will convert some of the soluble insulin into protamine insulin, although many authorities now sanction putting the soluble insulin and protamine zinc insulin in the same syringe. In view of the possibility of severe and protracted hypoglycæmic reactions occurring after the injection of protamine zinc insulin, it is wise not to inject more than 50 units a day, although doses up to 100 units or more are sometimes given in one injection.

In all cases adjustment of the carbohydrate content of the four meals should be made to suit the individual requirements of the patient, as shown by his blood sugar readings or the occurrence of glycosuria. With a single daily injection of protamine zinc insulin there is no necessity for the meals to be eaten at definite times. Further, Hanssen (44), Joslin (45) and Sheriff and Copp (46) have shown that the enlarged and probably fatty liver, which is met with in some cases of diabetes, appears to diminish more effectively with protamine than with soluble insulin.

Disadvantages of the Protamine Insulins

Certain drawbacks to the use of the protamine insulins have been noted. Thus (*a*) protamine insulins, owing to their slow rate of absorption, are of no use in the immediate treatment of hyperglycæmic coma. They can never therefore completely replace soluble insulin. (*b*) The rate of absorption of protamine zinc insulin is apt to be variable. (*c*) In no severe case is it likely that one daily injection of protamine zinc insulin alone will prove sufficient. A fresh complication is therefore added, as the patient has to inject two varieties of insulin simultaneously. (*d*) Severe and protracted hypoglycæmic reactions may occur with protamine zinc insulin, with only slight warning symptoms. The patient may pass straight into coma, and this may occur during the night, or he may first become drowsy with vague mental confusion, lassitude and hunger. These are especially liable to occur if a dose of more than 40 to 50 units is given. Exercise is liable to produce hypoglycæmia in patients taking protamine zinc insulin. Before playing golf, for instance, Mosenthal and Mark (42) recommend that the patient should take a sandwich and a glass of milk. The treatment of the hypoglycæmia is also more difficult, and successive doses of dextrose, up to 100 grams, may be required before the patient finally recovers. (*e*) Apart from these disadvantages, which concern the patient, the preparation and standardisation of the protamine insulins present considerably more difficulties than is the case with soluble insulin.

Changing from Soluble to a Protamine Insulin

This should not be undertaken lightly, but only for some special reason, as detailed above. Such attempts may prove

unsuccessful, and the patient is glad to return to the soluble insulin which has served him so well before.

The change from soluble to a protamine insulin usually involves alterations in the diet as well as in the insulin. The amount of carbohydrate at breakfast has to be reduced and that at tea increased. If protamine zinc insulin is being used in a severe case of diabetes the usual morning dose of soluble insulin should be injected, together with two-thirds the amount of insulin previously required during the remainder of the day. This amount is given as protamine zinc insulin at the same time as the soluble insulin. The dose of protamine zinc insulin is then gradually increased until the sugar is controlled during the afternoon, evening and night. Later it may be possible to reduce the amount of soluble insulin which is required to control the after-breakfast blood sugar rise. A few days should be allowed between each alteration in the protamine zinc insulin dose, as the optimum effect may not be obtained immediately.

In one of our cases the patient was stabilised on a diet of C. 242 g., P. 99 g., F. 68 g., Cals. 1,976, with soluble insulin 60 units before breakfast, 30 units before lunch and 60 units before dinner. He was then again stabilised on a diet of C. 272 g., P. 105 g., F. 68 g., Cals. 2,120, with a morning injection of protamine zinc insulin 88 units and soluble insulin 60 units.

In the mild cases of diabetes a single morning or evening injection of protamine zinc insulin may be sufficient to replace a morning and evening injection of soluble insulin. There is often a tendency to irregularity of action of the protamine insulins, so that the urine tests may vary more from day to day than they do with soluble insulin. In very severe cases acidosis may occur during the initial stages of the change over; this should be corrected at once by an injection of soluble insulin and the oral administration of dextrose.

Globin Insulin

This is a water clear solution, composed of globin, derived from hæmoglobin, insulin and a trace of zinc. It was first prepared by Reiner et al. (47), and was introduced as a preparation intermediate in rapidity and duration of action between soluble and

protamine zinc insulin. It is put up in two strengths, 40 and 80 units per ml. The effect is said to begin about four to six hours, to be maximum at about eight hours and to fade away before twenty-four hours after injection. Although some favourable reports have appeared, globin insulin has not been generally accepted as of great value in the treatment of diabetes. Mosenthal (48) found it of use for patients whose blood sugar rises after eating only, and not during the night when fasting. Malins (49) used it in 36 cases, obtaining good control of the diabetes in 10. He found that the liability to hypoglycæmic reactions was its greatest drawback. Eaton (50), as the result of treating 41 cases with globin insulin, considered that the freedom from local reactions is a point in its favour.

In our experience local reactions may be so troublesome as to necessitate abandoning the globin insulin. Further, there is often hyperglycæmia after breakfast and hypoglycæmia during the afternoon, and we may conclude that globin insulin has not fulfilled its early promise as an intermediary type of insulin. In patients not controlled with a single dose of protamine zinc insulin, it is probably best to give protamine zinc insulin and soluble insulin before breakfast, either mixed in one syringe or by separate injections.

Lipocaic

Dragstedt et al. (51) reported on a new pancreatic hormone concerned with normal transport and utilisation of fat. They obtained a substance which they called " lipocaic " in alcoholic extracts of beef pancreas. They consider that the development of fatty liver, atheromatosis and arteriosclerosis, which appeared in depancreatised dogs maintained for six or nine months on insulin, was due to lipocaic deficiency. Dragstedt (52) considers that the active substance in the pancreas is probably not choline, and, as it is not present in the external secretion of the gland, it probably represents a specific internal secretion distinct from insulin. Four cases of hepatomegaly in human beings suffering from diabetes mellitus, but well controlled with diet and insulin, were successfully treated with lipocaic. It may be that presenile arteriosclerosis occurring as a complication of diabetes is also

related to lipocaic deficiency. The clinical use of pancreatic extracts containing lipocaic is still in the experimental stage.

After-treatment in Diabetes

The treatment which has just been described may be considered as the preliminary or stabilising treatment in diabetes : it consists in establishing an adequate dietary for the patient, so that the urine remains sugar free and the blood sugar is within normal limits.

As, however, the after-treatment is equally important, there are several points on which advice must be given before the patient leaves the shelter of constant medical supervision.

It is essential, first, that he shall understand the nature of the disease from which he is suffering and the fundamental principles of its treatment. These can be explained in simple terms intelligible to the layman. If he is taking insulin he should also be instructed in the method of its administration before he resumes his ordinary home life. Advice should be given on the following points :—

The Supply of Insulin. This is sold in small rubber-capped bottles. The insulin should be stored in a cool, dark place : the soluble insulin will keep for several months, but should only be used if perfectly clear. Twenty units of soluble insulin are contained in each ml., but a double strength (40 units per ml.), and still greater strengths of insulin (80 and 100 units per ml.), are available. The strengths and directions for the use of the protamine insulins are given above on p. 119.

The Insulin Syringe. A specially graduated syringe is simple to use. The graduations are in units, each division being $\frac{1}{20}$ ml. The syringe may be kept in a metal case filled with proof spirit, which should be renewed each week to prevent the needle rusting. A stainless steel needle, size 16 to 18, may be employed. With such a syringe the necessity of sterilisation by boiling is abolished. As the rubber cap of the insulin bottle is apt to blunt the needle, it is preferable to employ two needles, one of which is used for piercing the rubber cap and filling the syringe and the other for injecting the insulin. The former may be of larger gauge than the latter, and can be kept separately in a small glass-covered vessel containing spirit. The needles should be sharpened from

time to time on a curved piece of carborundum. The injections may be made into the arms or thighs, but the skin on the arms cannot be pinched up on inserting the needle if the patient gives his own injections. The site of the injections should be changed daily, not more than one injection being given at the same site for several weeks, otherwise absorption may be delayed and sepsis occur. If a local reaction, either urticarial or hæmorrhagic, results from the insulin injection, another brand should be tried, until one is found which does not cause it. The reaction is due to the protein in the insulin, as some brands are made from beef and others from pig pancreas.

The necessity for taking carbohydrate food within half an hour after a dose of soluble insulin must be emphasised, but the diet and dosage of insulin will have been worked out before the patient begins his after-treatment.

Urine Examinations. A specimen of urine should be examined on retiring at night and on rising in the morning. The patient should be taught to test the urine by Benedict's method. He should also test a specimen daily with ferric chloride for diacetic acid, and if this is present he should at once inform his doctor. Rothera's test is so delicate that minute quantities of acetone bodies may often be found, which are not of serious significance. If both specimens remain sugar free, the insulin dosage should be reduced unit by unit, or a little more carbohydrate added to the food until sugar just appears. The object is to keep the urine sugar free and at the same time reduce the insulin or increase the carbohydrate as much as possible in accordance with any improvement in the sugar tolerance.

Hygiene. Owing to the liability to gangrene the diabetic should pay particular attention to his feet, washing them daily and drying them well. Wet socks and shoes should be changed as soon as possible. Constipation should be avoided and, if necessary, a regular aperient taken. Any illness or infection tends to make a diabetic worse; thus with a common cold the insulin dosage will require to be increased. If, owing to gastro-intestinal disturbance or to a feverish illness such as influenza, the patient cannot take his diet, he must neither starve nor omit his insulin or he will probably go into coma. He should take fluids such as milk up to 3 pints a day, and orange juice with dextrose 1 oz. as

three of the feeds, and insulin should be administered before the dextrose feeds in sufficient amounts to control the glycosuria.

A blood sugar estimation should be carried out once a month in the early stages of the after-treatment, and later at progressively longer intervals. This is the only possible way of knowing that the blood sugar is within normal limits, as the renal threshold for sugar excretion may change in the same patient during treatment, as previously described.

Exercise. Patients who are using insulin should take regular exercise but should not indulge in violent exercise, as hypoglycæmic coma has been known to occur in such cases. Exercise lowers the blood sugar in patients who are receiving an adequate supply of insulin, but raises it when the insulin dose is insufficient. The exercise should therefore be taken on a falling blood sugar, *i.e.*, within three hours of an injection of soluble insulin.

Symptoms of Hypoglycæmia. The patient should be told clearly the significance of hypoglycæmia, and the symptoms by which he may recognise its onset. He should always carry about two or three lumps of sugar which he can eat if he feels any such early symptoms as sweating or faintness after an injection of insulin. It is a debatable point whether or not every patient who is having insulin should be given a deliberate overdose during the initial stage of treatment, in order to familiarise himself with the symptoms of hypoglycæmia, and the ease with which they can be abolished in the early stages by appropriate treatment. It is not our custom to do so.

Anæsthetics in Diabetes

With proper preparation anæsthesia can be safely induced in cases of diabetes. The anæsthetic employed may be local, spinal, gas and oxygen, ether and such basal anæsthetics as Pentothal Sodium, which are administered intravenously. Chloroform should never be used. When Pentothal Sodium is used to produce only a short anæsthesia, such as is required for an easy dental extraction, no special preparation or alteration in the diabetic routine is required. For longer anæsthesia with Pentothal Sodium, the patient should be prepared as described later. Even when operation is to be performed under a local anæsthetic, as in cataract, the patient should be prepared as if for a general

anæsthetic. The mental shock of the operation may precipitate coma if these precautions are not observed. It must be remembered that the administration of any anæsthetic, even to a normal person, will cause a rise in the blood sugar.

The patient must not be starved before the anæsthetic, but should have his last meal three to four hours before the operation. Two and a half hours before the anæsthetic he should be given 30 units of insulin, and half an hour later, by mouth, 2 oz. of dextrose dissolved in 6 oz. of water. The patient should return to the medical ward after the operation and, if the diabetes is severe, he will require the same careful attention as would be given to a patient on the verge of coma. The bed should be warmed by an electric cradle. During the first twenty-four hours, 150 to 180 g. of dextrose must be given by mouth or intravenously. Thus 2 oz. of dextrose dissolved in 6 oz. of water should be given by mouth three times during the next twenty-four hours with 10 to 20 units of insulin injected half an hour before each dose. If the patient vomits the first dose, 20 ml. of 10% solution of dextrose (2 g.) should be given intravenously, and repeated if the patient continues to vomit, giving 1,500 ml. during the twenty-four hours in three injections of 500 ml., each preceded by 20 units of insulin. Before giving the first injection of insulin the blood sugar and alkali reserve should be determined, and no insulin injected unless the blood sugar is above 120 mg. per 100 ml. If the blood sugar cannot be determined a specimen of urine should be obtained, if necessary by catheter, and no insulin given if it is sugar free. In addition, a patient must receive 4 pints of fluid in all during the first twenty-four hours, either by mouth, or intravenously if he vomits. The next day he will probably be able to take by mouth citrated milk feeds, 4 to 6 oz., every three hours, with 1 oz. of dextrose in three of the feeds. Half an hour before these dextrose feeds 10 to 20 units of insulin should be injected. Meat extracts may also be given and water to bring the fluid intake up to 4 pints in twenty-four hours. The urine should be tested before each injection of insulin for sugar and acetone bodies. On the third and fourth days additions may be made to the diet, such as orange juice, thin toast, jellies, and steamed fish. The patient can then be worked up to his previous diet and the insulin dosage adjusted.

Diagnosis and Treatment of Coma in Diabetes

Coma occurring in the course of diabetes may be due to (1) ketosis or (2) hypoglycæmia. There may be some difficulty in differentiating between these two conditions, but before considering the differential diagnosis it would be well to describe them.

Coma due to Ketosis. The best way of describing this subject is in a manner approaching that in which the practitioner or house physician would meet it. He is summoned to the bedside of a person lying unconscious, and either through a previous history of diabetes, or through a careful clinical and biochemical investigation, he comes to the conclusion that a diagnosis of diabetic coma is probable. From the clinical point of view one might state briefly that the patient in diabetic coma will show air hunger, the breath will smell of acetone, and this is particularly noticeable if the patient is in bed in a small room ; all the muscles are equally flaccid, the deep reflexes are depressed and the plantar responses are flexor ; in 65% of cases the pulse rate is over 130 per minute ; there is also often a leucocytosis of from 15,000 to 50,000 white cells, even apart from any recognisable infective conditions (Joslin (53)). Another point which is considered as being of great diagnostic importance is the softness of the eyeballs ; this may readily be determined on palpation and is a constant feature. Before the patient becomes comatose the symptoms are often abdominal ; nausea, vomiting of dark brown fluid containing altered blood, and diffuse abdominal pain with widespread tenderness and muscular spasm. The clinical picture may closely simulate an acute abdominal catastrophe.

The importance of a thorough general examination is great, and there can be no doubt that one of the most helpful signs in diagnosis is the temperature. In a case of straightforward diabetic coma this is always sub-normal, and it is a safe rule to work on that, if the temperature is raised or is even normal, the coma is complicated by some second factor or is due to some other condition such as meningitis. Thus, examination of the chest may show that the patient has advanced pulmonary tuberculosis, or it may be that some obvious septic infection, such as gangrene of the extremities or a carbuncle, is present. The discovery of any of these is of great importance, since it will

indicate to the physician that he will probably have to use much larger doses of insulin than in an ordinary case, and he need not fear, to anything like the same extent, the onset of hypoglycæmia. If a superficial septic infection is found, it is advisable to treat this at once in the usual manner, and to render it as clean as possible. Many physicians are of the opinion that some form of septic focus will always be found in diabetic coma.

One final word of advice may be given about the possibility of mistaking cerebral lesions such as meningitis or cerebral hæmorrhage for diabetic coma. Meningitis and cerebral hæmorrhage are associated with hyperglycæmia and glycosuria, and a certain amount of ketosis may be present. They are excluded on a careful clinical examination. The possibility should always be borne in mind of encountering a diabetic patient with coma from some other cause, such as hemiplegia or cerebral hæmorrhage. The subject of hypoglycæmic coma will be dealt with later.

The next step is to obtain specimens of blood and urine, the latter by passing a catheter. The blood should be sent to the laboratory for estimation of blood sugar, blood urea and alkali reserve, the latter being of great value from a prognostic point of view, since it is a more delicate indication of ketosis than the simpler tests of Rothera and Gerhardt. It will usually be found that the urine contains large quantities of sugar, turning Benedict's reagent a deep orange-red colour, and the Rothera and ferric chloride tests will be strongly positive ; on rare occasions, however, sugar is not present. The urine should also be examined for albumin and for casts, and the amount passed every twenty-four hours measured.

The statement that diabetic coma can be found in a patient who is sugar free may be read in most text-books, yet no account of how this occurs is given. If the patient is sugar free, how may the diagnosis of diabetes be made ? We have seen one case of coma under these conditions. The patient had glucose in the urine and was suddenly deprived of carbohydrate in his food. His urinary sugar disappeared, but the fat metabolism became so deranged that he went into coma.

There is another rare group of cases where the urine of the comatose patient contains no acetone bodies ; further, in some of these the breath may smell of acetone, and acetone bodies be

found in the blood and cerebro-spinal fluid. In this type of case there is failure of renal function, the volume of urine passed in the twenty-four hours is generally low, and casts and albumin are present. The blood urea is also usually raised. Cases approximating to this type have been recorded by several observers, including Begg (54), Payne and Poulton (55), Oliver (56) and Graham (57) in England, and Rosenbloom (58) and Paddock (59) in America.

Without waiting for the blood sugar result, 40 to 60 units of insulin should be given subcutaneously at once, together with 2 oz. (57 g.) dextrose dissolved in 1 to 2 pints (568 to 1,136 ml.) of water by mouth, or 20 ml. of a 10% solution of dextrose (2 g. or gr. 30 dextrose) in normal saline administered intravenously, if the patient cannot swallow or if the blood pressure is very low. Some observers have recommended the administration of insulin by the intravenous route, maintaining that if the circulation is very depressed or the blood very viscous, a more immediate effect will be obtained. Whilst it may be desirable to adopt this procedure in very severe cases owing to a great deal of collapse, our experience has shown that the intravenous administration has no advantage over the subcutaneous method. Whilst sodium bicarbonate used to be given in large quantities, it has been found that just as good results are generally obtained by using insulin and dextrose alone. Starr and Fitz (60) have, however, shown that in some cases the acidosis may be due in part to acid substances other than ketone bodies, which are not affected by insulin. As a routine, therefore, it is advisable to administer sodium bicarbonate gr. 60 by mouth every six hours. The dextrose should be administered in as large a quantity of water as is conveniently possible, as this tends to combat general dehydration. The patient is usually dehydrated by the polyuria, and 8 oz. of water should be given by mouth every hour. There is no doubt that patients can be brought round from severe diabetic coma by means of large doses of insulin without the simultaneous administration of dextrose. Thus Bailey (61) successfully treated a comatose patient with 1,820 units of insulin in seven hours without the use of dextrose, and in another case 2,272 units were given in twenty-four hours, during a phase of insulin-resistance, before consciousness was regained. Root and Carpenter (62) go further than this and

suggest that dextrose may actually do harm. It is said that as the respiratory quotient is not raised the dextrose does not increase carbohydrate combustion, and that dextrose may damage the liver or lead to anuria. The importance of giving large amounts of insulin during the first three hours is emphasised by the figures from Joslin's Clinic, which were analysed by Root (63). In a series of 525 cases recorded by Joslin et al. (64) treated with an average dose of 83 units of insulin during the first three hours, the mortality was 11%; in a subsequent consecutive series of 123 cases treated with an average of 216 units of insulin in the first three hours, the mortality was 1·6%. Almy et al. (65), writing later, state that the most urgent requirements in diabetic coma are insulin and isotonic sodium chloride solution, and they also advise the administration of dextrose. The special point in their *régime* is the half-hourly administration of 25 units of insulin, on the supposition that small doses metabolise more carbohydrate per unit than do large ones.

While these investigations are being conducted, the report on the blood sugar should be at hand, although, of course, in every case this may not be possible. If the patient is in very deep coma, the blood sugar will probably be very high—in the region of 500 mg. per 100 ml., whilst the alkali reserve will be greatly reduced, possibly to a figure below 30 ml. CO_2 per cent. In such a case it would be advisable to give another 50 units of insulin immediately, together with another 2 or 3 oz. of dextrose in a pint of water by mouth, or 20 ml. of a 10% solution of dextrose in normal saline intravenously, if the patient cannot swallow. If, however, the patient is only lightly in coma, the blood sugar being only moderately raised and the alkali reserve over 35 ml. CO_2 per cent., then it will not be necessary to give more insulin at once. If blood sugar estimations are not available, then urine specimens, taken every three hours just before the insulin is injected, will serve as a control, and no further insulin should be given until the first three hours have elapsed.

There will now be a period of three hours before any more specific anti-diabetic steps can be taken, and this period should be employed in attending to the patient's general condition. If the patient is cold and collapsed, he should be kept warm either by means of hot water bottles, great care being taken that these are

not so hot as to burn the unconscious person, or by means of a cradle fitted with electric lights. If the latter is employed, it is essential to keep a careful watch on the temperature in the bed. It should not be allowed to rise above 99° to 100° F., and one light will usually be found sufficient.

The blood pressure has already been referred to ; if this is low, it indicates that the patient is suffering from severe shock and is in imminent danger of dying from circulatory failure. Thus, a blood pressure of below 70 mm. Hg. systolic points to a very grave prognosis, and steps should be taken immediately to combat this side of the disease. There are several means at the disposal of the physician : a blood transfusion may be given, and a pint of blood of the right group introduced into the patient's circulation ; or a pint of warm normal saline may be transfused slowly intravenously or subcutaneously. Graham (57) has suggested that a hypertonic saline solution should be administered intravenously ; 1 pint (568 ml.) of a 1·2% solution of sodium chloride may be given. The increased concentration of the blood is shown roughly by the difficulty with which it is withdrawn into a syringe from a vein. A more accurate indication is given by the hæmoglobin percentage. If it is above 100% intravenous saline should be given and repeated in four hours' time, if still indicated.

It has been claimed by some observers that the underlying cause of circulatory failure is loss of capillary tone, thus leading to stagnation in the capillaries, followed by concentration of the blood. To counteract this ephedrine hydrochloride, gr. $\frac{1}{2}$ in 1 ml. ampoule, may be given hypodermically ; in some cases pituitrin, $\frac{1}{2}$ ml. at two-hourly intervals, has been employed. The disadvantage of the latter procedure is that the blood sugar is immediately raised owing to stimulation of the glycogenolytic functions of the liver. In the case of cardiac and respiratory failure, 1·5 to 5 ml. of Coramine (nikethamidum, B.P. Add.) should be given slowly intravenously, or strychnine, gr. $\frac{1}{30}$, subcutaneously.

Attention should be paid to the bowels, as patients in diabetic coma are often very constipated. It will usually be found necessary to administer an enema followed by washing out of the colon, this being effected by moving the patient on to his side. A word of warning should be given about administering enemas to very collapsed and weak patients. If the case is a very severe

one, it will probably be wiser to postpone any vigorous measures of this type until the patient has recovered consciousness. If the results of the enema have been satisfactory, ½ pint (284 ml.) of normal saline should be introduced into the rectum in the hope that it will be retained and absorbed.

John (66) emphasises the importance of gastric lavage, unless the patient is too collapsed to endure this procedure. The stomach often contains undigested food, and lavage with warm normal saline relieves abdominal distress and vomiting and lessens the strain on the heart. Further, the danger of the stomach contents regurgitating into the lungs is avoided.

By this time the first three hours following the injection of insulin will be at an end. If the patient has only been lightly in coma, it is possible that consciousness will have returned. This takes place in a manner very similar to a patient recovering from an anæsthetic. The respiration changes from the regular, long, sighing type to the irregular, noisy variety, the patient grunting at first, then groaning, and often shouting in a drunken manner. The limbs, after being in a state of extreme flaccidity, begin to stiffen and the arms are waved about, and gradually the patient becomes rational.

In any case, the bladder should be emptied, either by catheter, or if possible by the patient's voluntary action, and the urine tested for glucose and acetone bodies. If possible, blood should be taken and the sugar content and alkali reserve determined. If the patient has recovered consciousness or shows signs of doing so, and there are still sugar and acetone bodies in the urine, then 25 units of insulin may be injected and 1 oz. of dextrose given by mouth in 1 to 2 pints of water; but if the patient is still deep in coma and the urine loaded with sugar and acetone bodies, then a larger dose must be given. If it is possible to obtain the blood sugar figure and this is high, then 100 units of insulin may be given subcutaneously with proportionately larger doses of dextrose. Variations in the severity of the condition require gradation of the dosage between these limits. If the blood sugar shows little or no change from that on admission, and the patient is still deep in coma, 150 to 200 units should be given. It is unwise, however, to give such large quantities unless an accurate watch can be kept on the blood sugar.

This type of regimen is carried out until the patient recovers consciousness, specimens of urine being examined every three hours just before the next injection of insulin and the dextrose are given. In very severe cases it may be necessary to give as much as 2,000 units of insulin during the twenty-four hours, but with these large doses the physician must be for ever on the watch for a sudden fall in the blood sugar with the concomitant production of hypoglycæmic coma. If blood sugar estimations are not available, not more than 10 to 20 units of insulin should be given if the urine contains only a little sugar, as it is possible for the blood sugar to fall to 70 mg. per 100 ml. or less and the urine still to contain some sugar. It is very important, however, not to be too overawed by this danger, as it must be realised that many more patients die from unrelieved hyperglycæmia than from hypoglycæmia.

When consciousness has been regained and the patient is quite rational, he has to be treated with just as much care and thought as when in coma. It is possible that the urine may be sugar free and the blood sugar normal, and it is a common error to let the patient have a twelve hours' fast from this time onwards, it being the intention to give him a No. 1 diet on the following day. The observer is then often astonished to find that at the end of a twelve hours' fast the urine is loaded with sugar and acetone bodies and the blood sugar is up to some 400 or 500 mg. per 100 ml. The reason for this is not difficult to see when one considers what has been happening during the period of treatment. The patient who has just come out of coma and is sugar free is by no means in the same condition, from the metabolic point of view, as the non-comatose person who has been starved until he is sugar free. This difference can readily be demonstrated by letting them both starve for twelve hours. Whilst the non-comatose patient will remain sugar free, the patient who has recovered from coma will pour out sugar into his blood and urine.

The reason for this is that the patient in coma has been given large doses of insulin combined with dextrose, with the result that the glycogenetic function of the liver has been stimulated and the liver has become packed with glycogen. At the end of the period of treatment, when consciousness has been regained and possibly the urine is sugar free and the blood sugar normal, the liver is

loaded with glycogen and the diabetic condition only temporarily under control. Now if such a patient starves for twelve hours and receives no insulin, the liver discharges its glycogen in the form of glucose and the blood sugar immediately rises and sugar appears in large quantities in the urine. In addition to the return of glycosuria, acetone bodies appear in high concentration in the urine when the liver is depleted of glycogen, and the alkali reserve falls. If this is allowed to continue the patient will relapse into coma again. These results can only be explained by the fact that the diabetic condition becomes re-established and the system becomes flooded with carbohydrate which cannot be metabolised, owing to lack of insulin.

In the case of the non-comatose diabetic who has been starved until he is sugar free, the liver contains no glycogen, and consequently further starvation produces no change in the blood sugar. It follows, therefore, that even if consciousness has been regained in the comatose diabetic, it is necessary to continue the insulin in order to prevent the glycogen already deposited in the liver from being discharged into the blood stream.

The ideal course of events would be to put the patient on about a No. 3 diet, but it must be realised that the events of the last twenty-four hours have been a severe strain on the patient and he is not in a fit state, mentally or physically, to commence eating ordinary food, although he may be hungry. By far the best thing to do is to give the patient 6 to 8 oz. of milk every three hours, with ½ to 1 oz. of dextrose in three of these feeds during the twenty-four hours, and to give insulin three times a day before the milk and dextrose feeds, sufficient either to keep the urine sugar free, or, in the event of being unable to obtain blood sugar estimations, with just a trace of sugar in the urine. After twenty-four to forty-eight hours the patient's strength will have recovered, and he may be given a diet such as a No. 3 or 4, according to his general condition, appetite, etc.

As soon as this diet is instituted, ketone bodies will almost certainly appear in the urine, and frequently the Rothera and ferric chloride tests will give a very strong response indeed, quite sufficient in fact to cause the greatest alarm to those unaccustomed to managing this stage of the treatment. But experience has shown that there is no need to fear the onset of coma, and the best method

of dealing with the situation is to add ½ oz. of dextrose three times a day to the diet. This will usually be found sufficient to abolish the ketosis, and as time goes on this may gradually be reduced as the diets are worked up.

It is still advisable to continue collecting the urine in three-hourly specimens, commencing at 6 a.m. and omitting the 3 a.m. collection in order to allow the patient six hours' undisturbed rest. By this means it will be possible to find out at what period of the day sugar is excreted, and the insulin can then be given at the time it is most required.

From this period onwards the treatment of the diabetic follows the ordinary lines laid down, the extra dextrose being omitted from the diet as the acetone bodies disappear from the urine, and the diets gradually increased through the series until the basal requirement diet has been reached. As soon as the patient is on the progressive series of diets and the stage of immediate anxiety is passed, the physician should institute a very thorough search for a septic focus. This will include the ordinary methods of examination, and in addition such special methods as X-rays of teeth, sinuses, appendix, and gall-bladder.

The importance of albuminuria in diabetic coma, with concomitant increase in the blood urea and non-protein nitrogen, has been emphasised by many workers. It is stated by some that if the blood urea is very high then the prognosis is bad. In our experience, however, it is very common to find a blood urea of 70 mg. per 100 ml. or over, in ordinary, straightforward diabetic coma, and in our opinion this is not necessarily of grave prognosis. A combined nephritic type of case is recorded as described on p. 129, but this is usually associated with suppression of urine, albuminuria, and granular casts in the urine, in which case the outlook is very grave.

Coma due to Hypoglycæmia. This condition may arise during the treatment of a diabetic, either due to an overdose or prolonged action of insulin, or to the patient not eating his diet and still continuing the insulin, or to his taking too much exercise. Sometimes, during the treatment of coma, the patient may pass from hyperglycæmic coma to hypoglycæmic coma without recovering consciousness. This course of events can occur if the blood and urine sugar are not watched very carefully in the

manner described. The danger of this happening is the justification for giving dextrose with the insulin, as this acts as a buffer against hypoglycæmia, and in our opinion large doses of insulin should never be given to an unconscious patient without a simultaneous administration of dextrose intravenously. Should hypoglycæmic coma occur, an injection of 1 ml. of adrenaline should be given subcutaneously, followed, if necessary, by the intravenous administration of 20 ml. of a 10% solution of dextrose (= 2 g. or gr. 30 dextrose) in normal saline. These injections should be repeated every half hour until the patient recovers consciousness. In some cases as much as 200 g. of dextrose are required. When the patient can swallow he should be given 10 g. of dextrose by mouth every hour for several hours, especially if the hypoglycæmia is due to a protamine insulin, as relapses into hypoglycæmia may otherwise occur.

Early Symptoms of Hypoglycæmia

Usually, but not invariably, these do not appear until the blood sugar falls to 50 mg. per 100 ml. Thus, with patients who for long have become accustomed to a high blood sugar content, hypoglycæmic symptoms may appear when the sugar has only fallen to 250 mg.

The condition is, perhaps, analogous to the state of the patient who has become accustomed to a high blood pressure, and is uncomfortable if it is lowered, although only to a figure considerably above the normal for a healthy person of the same age.

On the other hand, hypoglycæmic symptoms may not be noticed until the blood sugar falls to 32 mg. per 100 ml., especially if the patient is receiving injections of a protamine insulin (see p. 120).

The early symptoms are variable and include sweating, apprehension, flushing, pallor, a sense of constriction around the waist, and coldness of the extremities. These manifestations may be followed by collapse, convulsions and delirium. The symptoms usually first appear about two hours after the injection of insulin, but may not be noticed for as long as twelve hours. At the earliest warning the patient should take two lumps of sugar, a few sticks of barley sugar or 5 g. of dextrose. An injection of 1 ml. of 1/1,000 adrenaline hydrochloride, or 1 ml. of pituitrin intramuscularly,

is of value if there remains any glycogen stored in the liver or muscles. As a prophylactic measure it is important to remember that an adequate carbohydrate meal must always be given at night after the evening dose of insulin in order to prevent hypoglycæmia developing during sleep, and it is wise for the patient to take 5 g. dextrose after a meal if he is going to drive a car.

Spontaneous Hypoglycæmia

The over-production of insulin associated with the signs and symptoms of hypoglycæmia was first described by Harris (67) in 1924 under the term hyperinsulinism. Since that time a considerable literature has grown up around the subject and a number of authentic cases have been described. The symptoms appear to be the same in all cases. In early or slight cases the patient notices severe hunger, rapidly relieved by food or carbohydrate. Sweating, noises in the ear, convulsions, loss of memory, aphasia, weakness and giddiness leading to actual unconsciousness, are encountered in the more severe type of case. Cardiac symptoms predominate in some cases, either anginal in type or resembling those of the effort syndrome. The neuropsychiatric aspects of hypoglycæmia have also attracted attention. Romano and Coon (68) described a patient who suffered from episodes of uninhibited emotional behaviour, which were cured by removal of an islet tumour. Hill et al. (69) have found that people with cerebral dysrhythmia, as shown by the electro-encephalogram, and this comprises about 10% of the general population and 26% of psychoneurotics, are very susceptible to a fall of the blood sugar, even to normal fasting levels. Abnormalities in behaviour in such cases may result from cerebral dysrhythmia, with a blood sugar as high as 100 mg. per 100 ml. A post-mortem examination, in cases where a fatal issue has supervened, shows a tumour of the pancreas of the islet cell type. In some cases the tumour has been found to be localised and removal has been associated with complete recovery. Smith (70) records a case in which a nodule of aberrant pancreatic tissue was found on the duodenum. Removal of the nodule improved the symptoms of hyperinsulinism. Conn (71) says that 80 to 90% of all cases of spontaneous hypoglycæmia fall into three groups, functional hyperinsulinism,

organic hyperinsulinism, and hepatic disease. The dextrose tolerance test, performed after a standard preparatory diet, helps to differentiate. In functional hyperinsulinism the fasting blood sugar is usually normal, whereas in organic hyperinsulinism the fasting blood sugar is low. In both cases the sugar tolerance curve is of a low type, and if prolonged for three or four hours it may fall sufficiently to produce hypoglycæmic symptoms. In hepatogenic hypoglycæmia the fasting blood sugar is low, but the curve shows a high plateau, similar to that seen in diabetes mellitus. The majority of cases of spontaneous hypoglycæmia are due to functional hyperinsulinism, but it should be remembered that severe or even fatal hypoglycæmic coma may occur during the course of Addison's or Simmonds' disease.

Treatment. An exploratory laparotomy is usually advisable in organic hypoglycæmia. The alternative is frequent feeds, rich in carbohydrate, together with the administration by mouth of ephedrine hydrochloride gr. $\frac{1}{2}$ t.i.d. In some cases of hepatogenic hypoglycæmia operation will reveal an infected gall-bladder. Alternatively a diet rich in protein and carbohydrate should be given. In functional hyperinsulinism it is advisable to avoid diets rich in carbohydrate, as the post-prandial rise in blood sugar is followed by a secondary fall, owing to increased insulin production resulting from the stimulation of the carbohydrate. Better results are obtained with a diet containing 120 to 140 g. of protein, 50 to 75 g. of carbohydrate, and 100 to 130 g. of fat. If necessary an extra protein meal should be given at bed time.

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CHAPTER V

THE LIVER

Investigation of the Pigmentary Functions of the Liver

Bilirubin and biliverdin are formed from hæmoglobin derived from broken down red cells. It will be remembered that bile pigments are converted in the intestine into a number of substances, the most important of which are urobilin and its precursor, urobilinogen.

These changes are brought about by bacteria. Urobilinogen and urobilin are absorbed into the circulation, and are picked out by the liver cells. Some doubt exists as to the fate of urobilin and urobilinogen, but they are probably re-excreted into the bile as bile pigments, biliverdin and bilirubin. A very small amount of urobilin is excreted normally into the urine, and special tests are required to demonstrate its presence. Damage to the liver cells causes them to lose this property of converting urobilin into bile pigments, with the result that this body accumulates in the blood, and is excreted into the urine in easily demonstrable amounts. This change precedes the appearance of actual bile pigments in the urine by a considerable time. Thus tests for urinary urobilin are quite strongly positive in the pre-icteric stage of acute catarrhal jaundice.

Fouchet's Reaction for Bile Pigment in Blood (1). To 3 drops of serum on a white porcelain surface, 3 drops of the reagent (20 ml. H_2O, 2 ml. of 10% $FeCl_3$, 5 g. trichloracetic acid) are added. A white coagulum results from the precipitation of the serum proteins by the trichloracetic acid contained in the reagent. If the reaction is positive the coagulum turns a greenish-blue colour, reaching a maximum in about twenty minutes. This test is positive with dilutions of bile pigment up to 1 in 60,000.

Tests for Urobilin and Urobilinogen in Urine. *Ehrlich's Aldehyde Reaction* (2). To 5 ml. of urine 2 drops of a 3% solution of paradimethylaminobenzaldehyde in 50% HCl are added. If the test is positive the resulting mixture becomes deep red.

Occasionally it is necessary to warm the solution to bring about the reaction, which may take a few minutes to develop. This test, when positive, demonstrates the presence of a pathological amount of urobilinogen.

This colour reaction was applied by Watson (3) to the quantitative determination of the sum of urobilin and urobilinogen in both urine and fæces. In jaundice due to stone, no increase in the urinary urobilinogen was found except in the presence of such complications as acute cholecystitis or cholangitis, biliary cirrhosis incident to persistent biliary obstruction, or severe anæmia. Small quantities of urobilinogen were usually found in the fæces of cases of jaundice due to stone, presumably because of incomplete obstruction. In hæmolytic jaundice, increases in the amount of urinary urobilinogen were believed to be associated with a disturbed function of the liver and were not necessarily caused by an increased destruction of blood. A marked increase in the output of urobilinogen in the urine was observed in diffuse hepatic disease. Although Watson recommends the quantitative analysis of urobilinogen in twenty-four-hourly samples of urine and fæces as a revealing test of hepatic function, some of the practical details of the test, *e.g.*, the collection of the representative twenty-four-hourly specimens of fæces, are not easy to carry out. In addition, there is considerable variation in the day-to-day production of urobilinogen, which limits the interpretation of the findings.

Schlesinger's (4) *Test for Urobilin.* Twenty ml. of urine are acidulated with acetic acid, and then the urobilin is extracted by gently inverting with 5 ml. of amyl alcohol. The amyl alcohol layer is pipetted off and a few drops of a 10% alcoholic solution of zinc chloride or acetate are added to it. If a green fluorescence develops the test is positive; in addition, the characteristic spectrum of the solution (a band in the green between *b* and F) can be observed. A positive reaction demonstrates an excess of urobilin.

The familiar tests, such as Gmelin's, Hay's and the iodine test will not be described.

Tests for Bile Pigments and their Derivatives in Fæces. *Schmidt's Test for Hydrobilirubin.* A small amount of fæces is rubbed up in a mortar with a concentrated aqueous solution

of mercuric chloride. The mixture is transferred to a shallow, flat-bottomed dish and is allowed to stand for six to twenty-four hours. The presence of hydrobilirubin will be indicated by a deep red colour being imparted to the particles of fæces containing this pigment. This red colour is due to the formation of a hydrobilirubin-mercury compound. If unaltered bilirubin is present in any portion of the fæces, that portion will be green in colour, due to the oxidation of bilirubin to biliverdin.

The van den Bergh Reaction. In view of the easily available literature (*e.g.*, McNee (5)), only the briefest summary of this important work will be given here. Jaundice, at the time of Virchow, was considered to fall under two headings, hepatogenous and anhepatogenous. Thus, in the hepatogenous variety the condition resulted from obstruction of the biliary tract, whilst in the second, anhepatogenous variety, the bile pigment was thought to originate in tissues other than the liver. Later, owing to certain experimental work which need not be detailed here, the possibility of anhepatogenous jaundice was excluded. Thus Eppinger (6) stated that all jaundice was obstructive, and in those cases where obvious obstruction could not be seen the seat of the trouble was said to be in the fine bile capillaries. Later, however, the work of Aschoff (7), and his co-workers, pointed rather to the original separation of jaundice into hepatogenous, or obstructive, and hæmolytic, or anhepatogenous. His work has completely changed the views on the formation of bile pigments from hæmoglobin. Thus it used to be thought that this change was effected by the polygonal cells of the liver, but in the newer conception it is held that these simply pass the pigment on from another group of cells to the lumen of the bile capillary.

The polygonal liver cells separate the bile capillaries from the blood stream, but between the polygonal cells and the blood stream we have a further series of cells described by Kupffer. Kupffer cells are found in the liver, spleen, and other parts of the body, and belong to what Aschoff calls the *reticulo-endothelial system.* In the modern conception, it is assumed, on certain experimental evidence, that it is in this system of cells that blood pigments are converted into bile pigments. It can thus be seen that hæmoglobin in the blood stream is converted into bile in

the Kupffer cells, which normally pass it on through the polygonal cells to the lumen of the bile capillaries. The function of the polygonal cells, therefore, is simply transmission, and they are not essentially concerned with production of pigments.

Whether actual bile pigments, or their precursors, are formed by the reticulo-endothelial system has not yet been determined. According to this theory, it is possible to have jaundice arising in one of three ways :—

1. Where obstruction of the bile tract prevents the escape through the natural channels. The bilirubin so formed will then be reabsorbed into the blood stream, to be subsequently excreted in the urine.

2. Where the polygonal cells lose their power of transmitting pigment to the capillaries, with the result that it will pass direct from the Kupffer cells to the blood stream. A similar series of events might arise if, through excessive blood destruction, more pigment were offered than the polygonal cells could pass through. Some would then be absorbed direct into the blood stream, and the rest would pass normally into the biliary passages.

3. Where there is a combination of these two conditions. That is to say, although the polygonal cells are damaged, a little bilirubin might pass through into the biliary passage, from which, owing to obstruction, it would be absorbed into the blood stream. In addition to this, there would be direct absorption from the Kupffer cells.

The essential point to be realised is that, in purely obstructive jaundice, the bilirubin has passed through the polygonal cells of the liver, but that it is quite possible to have icterus due to bilirubin which has not passed through these cells. As will be shown later, these two varieties of bilirubin can be identified by a chemical reaction.

Accordingly, McNee recommends the following classification of jaundice :—

Obstructive hepatic jaundice, in which there is a condition similar to Group I. in the above scheme.

Hæmolytic jaundice, corresponding roughly to Group II.

Toxic and infective hepatic jaundice, in which there is a combination of obstruction and direct absorption from the Kupffer cells similar to Group III.

In Group I., the bilirubin in the serum will be of the variety which has passed through the polygonal liver cells, whilst in Group II. it will not have been transmitted through them, but in Group III. both varieties will be present. It is obvious that if a reaction could be found to distinguish between these varieties of bilirubin, the differentiation of jaundice according to the above classification would be possible. This has been done by Hijmans van den Bergh (8), who employs Ehrlich's azo reaction. This consists in adding to the solution of bilirubin a diazonium salt in acid solution, causing the appearance of a purple compound, azo-bilirubin. The diazonium compound is provided by mixing a solution of sulphanilic acid in HCl with sodium nitrite. Van den Bergh noted that icteric sera, when treated in this manner, either gave an immediate colour, or else a very delayed reaction. He was able to assert that the first type of reaction was given by bilirubin which had passed through the liver cells, and that the delayed reaction resulted with pigment formed without the agency of the polygonal cells, *i.e.*, absorbed direct from the Kupffer cells. In cases of toxic and infective hepatic jaundice, there is usually a biphasic response, probably due to the presence of both types of bilirubin.

The following account is the revised method for performing the reaction (9): The examination must be made within about two hours of taking the blood, or paradoxical results are obtained. Ten to fifteen ml. of blood are collected in an oxalated tube. The latter is prepared by adding 0·2 ml. of 10% potassium oxalate solution, and evaporating to dryness during the process of sterilisation. The tube containing blood is centrifuged and the clear supernatant plasma is pipetted off.

Solutions. 1. A freshly prepared solution of Ehrlich's diazo reagent. This is made by mixing the two following solutions immediately before the test :—

A.	Sulphanilic acid . .	1 g.
	Concentrated HCl . .	15 ml.
	Distilled water . . .	1,000 ml.
B.	Sodium nitrite . . .	0·5 g.
	Distilled water . . .	100 ml.

The actual reagent is made immediately prior to the test by

mixing in the proportions of 25 ml. of solution A to 0·75 ml. of solution B.

2. Absolute alcohol or 96%.

The Direct Reaction. Three small tubes are set up (numbered 1, 2 and 3), and 0·25 ml. of oxalated plasma is pipetted into each. To the first tube, the control, 0·2 ml. of water is added, whilst into No. 3 a small flake of caffeine-sodium salicylate is dropped, which has been found to accelerate the development of the colour change. After the salt has been dissolved by shaking, 0·2 ml. of fresh diazo reagent is added, when a prompt and definite reaction takes place, shown by the production of a purple tint. When this colour has attained its maximum, 0·2 ml. of the diazo reagent is added to tube No. 2, and any alteration in colour is compared with the fully-developed reaction in tube 3.

One of three events may occur in tube 2 :—

An immediate direct reaction. This begins at once and reaches a maximum in ten to thirty seconds. The mixture turns to a bluish-violet colour, the intensity varying with the concentration of bilirubin.

A direct delayed reaction. Here a reddish colour, deepening to violet, begins to appear in about fifteen minutes.

A direct biphasic reaction. In this type of response a reddish colour appears immediately and takes much longer to deepen into a violet hue.

The Indirect Reaction. If neither the first nor second response is obtained the indirect reaction should be proceeded with. To 1 ml. of plasma 0·5 ml. of diazo reagent is added, and after a minute or so 2·5 ml. of 96% alcohol and 1 ml. of saturated aqueous ammonium sulphate solution are added. After thorough mixing, the tube and contents are centrifuged and the supernatant clear fluid can be used for quantitative colorimetric analysis. If the colour is too deep it may be diluted with a mixture of two volumes of alcohol and one volume of water.

Quantitative Reaction. The indirect reaction may be converted into a quantitative process by matching against a standard of ferric thiocyanate in ether. This solution is made up as follows :—

Dissolve 0·1508 g. of ammonium iron alum in 50 ml. of concentrated HCl and add water to 100 ml. The resulting solution is quite stable, and is about 1 in 320 N. To 10 ml. of this solution

add 25 ml. of concentrated HCl, and water up to 250 ml. This gives a dilution of 1 in 8,000 N, which keeps for six months. To 3 ml. of this stock solution an equal volume of 20% potassium thiocyanate solution is added, and the resulting mixture is well shaken and extracted with 12 ml. of ether. The ethereal extract is used as the standard and the solution obtained in the indirect test is matched against it. This standard corresponds with a concentration of 1 in 32,000 N of bilirubin, *i.e.*, 1 in 200,000 azo-bilirubin. An indirect test, containing the same amount of colour as the standard, will contain 4 units of bilirubin, since the plasma is diluted approximately four times in this reaction.

Van den Bergh and Müller now recommend the following as a standard solution: 2·161 g. of anhydrous cobaltous sulphate are dissolved in 100 ml. of water, thus giving a solution which corresponds in colour with an azo-bilirubin solution of 1 in 200,000, *i.e.*, one unit. The salt must be anhydrous, and the standard solution should be checked against the one already described, *i.e.*, ferric thiocyanate, and must be stored in the dark.

An alternative method for performing the van den Bergh reaction is as follows :—

Method of King, Haslewood and Delory (10)

Principle. The red colour produced by bilirubin in the presence of diazotised sulphanilic acid is compared colorimetrically with an artificial standard containing methyl red.

Diazo-reagents. *Solution* A. Dissolve 1 g. of sulphanilic acid in 250 ml. of N hydrochloric acid and dilute to 1 litre with water.

Solution B. 0·5 g. of sodium nitrite in 100 ml. aqueous solution.

The reagent is freshly prepared, as required, by mixing 0·3 ml. of solution B. with 10 ml. of solution A.

Standard. *Stock Methyl Red Solution.* 0·29 g. of pure methyl red is dissolved in glacial acetic acid and diluted with acid to 100 ml.

Standard Methyl Red Solution. One ml. of the above stock solution, 5 ml. of glacial acetic acid, and 14·4 g. of crystallised sodium acetate are made up to 1 litre with water. This solution, at *p*H 4·63, contains 2·9 mg. of methyl red per litre, and matches

the colour obtained when 0·1 mg. of bilirubin is treated with diazo reagent in a final volume of 25 ml.

Method. *Quantitative Reaction.* 1 ml. of plasma or serum is treated in a centrifuge tube with 0·5 ml. of diazo reagent, 0·5 ml. of saturated ammonium sulphate, and finally 3 ml. of absolute alcohol. The mixture is stoppered, thoroughly shaken and filtered after a few minutes. The colour of the filtrate is matched against the standard in the colorimeter, using a green light filter.

Calculation. $\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 1{\cdot}6 =$ milligrams of bilirubin per 100 ml. plasma or serum

1 mg. of bilirubin = 2 units of bilirubin.

Direct Reaction. If the diazo reagent is carefully "layered" above the plasma and the tube allowed to stand for a few moments, a positive "direct" reaction is denoted by a red colour at the liquid junction.

The normal level of plasma bilirubin obtained by this method varies from 0·2 to 1·7 mg. per 100 ml., although most normal results are below 0·8 mg. per 100 ml. (Vaughan and Haslewood(11).)

Interpretation of the Results. According to McNee an immediate direct reaction indicates obstructive jaundice, whilst a delayed direct reaction points to a hæmolytic or non-obstructive form. The biphasic reaction is found in cases of toxic and infective jaundice, where both obstructive and non-obstructive processes are at work. While admitting the practical value of this classification, Rich (12), and Snell and Magath (13) have drawn attention to the fact that pure forms of jaundice seldom occur. In hæmolytic jaundice, for example, it is postulated that the increase in bilirubin in the plasma depends on a disturbed excretory function of the liver cells as well as on increased production of bilirubin. Snell and Magath (13) suggest that gross obstruction to the extra-hepatic bile passages never occurs without injury to the hepatic parenchyma, and conversely, a pure hepatic form of jaundice never occurs without injury or obstruction to the finer bile passages. Greene, Plotz and Localio (14) investigated the distribution of the two types of pigment in various cases of jaundice. In hæmolytic jaundice the pigment was of the kind which responded to the indirect reaction, while in obstruction due to stone or cirrhosis, the pigment in the plasma was mainly of the

direct reacting type, but was accompanied by an appreciable amount of the indirect reacting type. In hepatitis and in hepatitis with cirrhosis, both varieties of bilirubin were found in a proportion resembling that obtained in obstructive jaundice. Accordingly, a positive direct van den Bergh reaction may not distinguish obstructive jaundice from the non-obstructive type. It must be borne in mind that all sera giving a positive direct reaction also give a positive indirect reaction, although the reverse does not necessarily hold true. In a marked case of non-obstructive jaundice there may be as much as 30 units of bilirubin present in the serum. Many interesting facts have come to light since the advent of this reaction. Thus the bilirubin content of normal serum has been found to be between 1 in 1,000,000 and 1 in 400,000, or, taking 1 in 200,000 as a unit, the limits are 0·2 to 0·5 units although, as mentioned above, higher normal limits have been reported by some workers. The renal threshold value of bilirubin has also been worked out. Thus it is stated that bile does not appear in the urine until 4 units are present in the blood. In hæmolytic jaundice this relation does not hold, since it is quite possible to have between 5 and 18 units present in the blood with no bile in the urine. It is suggested that the bile is excreted in the form of urobilin in these cases. Perhaps one of the most important developments of this reaction is the recognition of cases of latent jaundice, in which there is sufficient bile to produce slight icterus, but not sufficient to produce bile in the urine. Pernicious anæmia usually belongs to this group. With regard to dissociated jaundice, *i.e.*, independent retention or excretion of bile pigments and salts, little light has yet been thrown by the reaction. Hæmolytic jaundice is certainly a dissociated condition, since the bile salts are unaffected. Retention of bile pigments alone has been observed in the later stages of catarrhal jaundice.

The Icterus Index. This is a useful method of following the variations in the intensity of jaundice from time to time in cases of jaundice.

The Bilirubin Excretion Test. The measurement of the excretion of bilirubin, injected intravenously, as a means of estimating hepatic function was originated by Eilbott (15). The technique described below is that of Soffer and Paulson (16).

A total amount of bilirubin equal to 1 mg. per kg. of body weight, but not exceeding 70 mg., is dissolved in 15 ml. M/10 sodium carbonate, which previously has been boiled and then cooled to 80° C. A control sample of blood is collected in a dry syringe to prevent hæmolysis, after which, with the needle *in situ*, the bilirubin solution is injected. Further specimens of blood are taken five minutes later and after four hours. Two ml. of plasma from the control and from the four-hour specimens are treated with 2 ml. of acetone and centrifuged, while 1 ml. of the plasma from the five-minute specimen is treated with 4 ml. of acetone. The supernatant fluids are filtered and promptly matched against suitable standards. These are prepared from stock 1 in 2,000 solution of potassium dichromate, which yields a colour identical with that of 1 mg. of circulating bilirubin per 100 ml. plasma. Suitable standards contain the equivalent of 0·9, 0·8, 0·7, 0·6, 0·5, 0·4, 0·3 and 0·2 mg. of circulating bilirubin per 100 ml. of plasma, and are made by diluting 9, 8, 7, 6, 5, 4, 3 and 2 ml. of the 1 in 2,000 dichromate to 10 ml. with water. When acidified with one drop of dilute hydrochloric acid, these standards are stable for some time and may be kept in sealed tubes.

Calculation. 200 × (mg. % bilirubin found in 4 hour filtrate — mg. bilirubin found in control filtrate) divided by (5 × mg. % bilirubin found in 5 min. filtrate — 2 × mg. % bilirubin found in control filtrate) gives the percentage of bilirubin retained.

Findings. Normally not more than 5 to 6% of the injected bilirubin should be retained at the end of four hours. An advantage of the test is that the liver is given a natural pigment, bilirubin, to excrete, although this substance is expensive and difficult to procure. It should be borne in mind that the test is inapplicable to patients with jaundice and is most useful in cases in which the bilirubin does not exceed 1 mg. per 100 ml. plasma. Several workers have reported favourably on the reliability and delicacy of the bilirubin test. Thus Soffer (17) found that the normal retention of bilirubin was exceeded in recovering cases of diffuse hepatitis in which the jaundice had subsided, as well as in cases of cirrhosis and malignancy. Sweet, Gray and Allen (18) concluded that the test was more delicate than the galactose tolerance, bromsulphalein and hippuric acid tests in the detection of hepatic

damage in cases of minimal or no symptomatic evidence of cirrhosis of the liver.

Investigation of the Antitoxic Powers of the Liver

The Oral Hippuric Acid Excretion Test. This test, which was devised by Quick (19), depends on the ability of the liver to conjugate benzoic acid with glycine. It has now been largely replaced by the intravenous test.

The Intravenous Hippuric Acid Test. In 1938 Quick et al. (20) showed that hippuric acid is synthesised in man following the intravenous injection of sodium benzoate. 1·77 g. of sodium benzoate is dissolved in 20 ml. of distilled water and injected slowly intravenously, taking five minutes over the injection. The bladder is emptied before the test and the urine is collected one hour after the injection. The hippuric acid is estimated in the urine. Normally 0·7 g. to 0·95 g. is excreted as hippuric acid in the first hour.

Method. Measure the volume of the hourly specimen. If this exceeds 125 ml. the sample should be slightly acidified with acetic acid and concentrated to about 50 ml. on the water bath before proceeding with the analysis. The specimen is then acidified with concentrated hydrochloric acid until acid to Congo Red. 1 ml. of acid is usually sufficient. The urine is stirred vigorously until the precipitation of hippuric acid is complete and then is allowed to stand for one hour at room temperature. The precipitate is filtered off on a small Buchner funnel using a weighed filter paper and is washed with a small quantity of water and allowed to dry in air. The hippuric acid thus obtained is weighed (to second decimal place) or titrated with N/5 sodium hydroxide using phenolphthalein as indicator. The total quantity of hippuric acid is obtained by adding to the weight of the precipitated acid that quantity which remains in solution. The latter fraction is calculated from the volume of the specimen and the known solubility of hippuric acid in urine (0·33 g. per 100 ml. urine). The result is converted to benzoic acid by multiplying by 0·68.

Findings. Mateer et al. (21 and 22) using this test, compared it with the oral method and found that the intravenous test is more delicate, yielding positive results (diminished hippuric acid excretion) in 85% more cases of liver damage than does the oral test.

The test is applicable to jaundiced and non-jaundiced patients. Dehydration, nephritis, or urinary tract obstruction may result in a low output apart from liver damage. Positive results are to be expected in hepatic cirrhosis and carcinoma. In cholelithiasis a positive result indicates liver damage and the necessity of a high carbohydrate, high normal protein, low fat diet before operation in order to reduce the operation mortality.

Investigation of the Metabolic and Hæmopoietic Functions

These methods have failed to yield information of much practical value, and will not be described in detail. While it is generally agreed that the galactose and lævulose tolerance tests are of limited value, there has been a tendency recently to modify or improve the original tests by the estimation of the amount of these sugars in the blood. In this way, using a modified galactose tolerance test, MacLagan (23 and 24) claimed to be able to differentiate between toxic and obstructive jaundice. The advantages of this technique are difficult to assess since it appears to have attracted little attention. The dextrose tolerance test is rarely used as a test for hepatic damage because of the many factors which are involved in the metabolism of this substance. The macrocytic anæmias associated with hepatic disorders are described on p. 329. Some of the more recent of these tests, *e.g.*, the colloidal gold test and the measurement of the prothrombin time are claimed to be more sensitive than the older tests. However, we do not feel justified in giving full descriptions of these tests at present, since further work is necessary before their exact value can be assessed. In consequence, these and other tests are but briefly mentioned below.

The Plasma Phosphatase. Roberts (25) was the first to note an increase in the plasma alkaline phosphatase in some cases of obstructive jaundice. A study of the level of this enzyme in plasma has been put forward as a means of distinguishing obstructive from non-obstructive jaundice. However, Green, Shattuck and Kaplowitz (26) as well as other workers, have failed to obtain clear-cut results in these two conditions. MacLagan (24) found that the alkaline phosphatase was usually over 35 units (normal 3 to 13 units/100 ml.) in cases of biliary obstruction, and in

infective hepatitis and arsenical jaundice usually below 25 units. When interpreting the results of the test, it should be borne in mind that the alkaline phosphatase may be raised in conditions other than liver disease, such as Paget's disease, rickets, hyperparathyroidism, osteomalacia, osteogenic sarcoma, and other primary skeletal tumours.

The Prothrombin Time. Wilson (27) found a reduction in the prothrombin time to be a more sensitive indication of liver damage than the bromsulphalein or hippuric acid tests. Using this method, Sweet, Gray and Allen (18) were able to demonstrate hepatic damage in 6 of their 8 cases of hepatolenticular degeneration. They employed the serial dilution one-stage technique of Allen, Julian and Dragstedt (28), in which the prothrombin times of various dilutions of the plasma are compared with the corresponding values of normal plasma. It is interesting to note that in 3 patients the abnormal prothrombin time was shown to be due to liver damage and not to vitamin K deficiency, since administration of an analogue of vitamin K left the prothrombin times unaffected. The response of cases of liver disease to vitamin K therapy has been investigated by Kark and Souter (29). Several types of response are observed depending on the condition of the liver. With intensive jaundice of doubtful diagnosis a rapid return of a lowered prothrombin level to normal after vitamin K therapy favours a diagnosis of obstructive jaundice. In cases of gross hepatic disease associated with a prothrombin level of less than 30 to 35% of normal, even after administration of vitamin K, the prognosis is considered to be grave. In other cases, where little or no response to vitamin K therapy is obtained, these authors recommend blood transfusion before and after surgical operation in order to increase the prothrombin concentration in the blood.

The Serum Colloidal Gold Test. Sweet, Gray and Allen (18) found the serum colloidal gold test of Gray (30) to be a sensitive test of hepatic dysfunction. Positive results were obtained with this test in 8 of the 9 cases of hepatolenticular degeneration studied. The precipitating action of serum on colloidal gold appears to depend on the balance of the precipitating action of the globulin and the protective action of the albumin. The positive results encountered in liver disease are attributed to a

qualitative rather than to a quantitative alteration in the globulin fraction. Mateer et al. (21) found 10% of false positive results in normal individuals, and concluded (22) that the test is not adapted to routine laboratory work. MacLagan (24) using a modified method found the test positive in 93% of cases of infective hepatitis and in 100% of cases of cirrhosis, and negative in 93% of cases of obstructive jaundice.

The Takata and Ara Reaction. This test, which was originally introduced as a means of differentiating between pneumonia and bronchopneumonia, depends upon the precipitation of serum by varying strengths of perchloride of mercury solution. It is not now thought to depend upon the amount of globulin present. The test (31) may be of value in the diagnosis of hepatic cirrhosis. Although Kirk (32) reported positive results in 315 out of 375 cases collected from the literature, he concluded that the reaction is not diagnostic of cirrhosis of the liver. Henderson and Splatt (33) consider the Takata-Ara reaction is likely to be positive in over 90% of cases of advanced cirrhosis hepatis, but negative in early cases. In 4 cases of secondary carcinoma of the liver it was strongly positive in 1, weakly positive in 2, and negative in 1. In 14 cases of cholelithiasis it was negative in 12, and weakly positive in 2. In jaundice the test will probably be positive in non-obstructive and negative in obstructive cases. They conclude that it is practically valueless as a test of liver function, but that it is a useful confirmatory test for parenchymatous liver disease. Britton (34) using a modified technique found in infective hepatitis a positive reaction during the icteric stage, varying in intensity with the severity of the liver damage and the course of recovery. Strongly positive reactions were obtained in advanced cirrhosis of the liver, in hæmochromatosis, subacute yellow atrophy, congenital syphilis of the liver and in Pick's disease. False positive and false negative reactions may occur. He concluded that the test is a simple and useful accessory to the clinical diagnosis and prognosis of parenchymatous liver damage.

Investigation of the Global Capacity of the Liver to eliminate Foreign Substances

The Bromsulphthalein Test. Rosenthal and White in 1924 (35 and 36) proposed a liver function test based on the ability of

the liver to remove bromsulphthalein (phenoltetrabromphthalein sodium sulphonate) from the blood.

Method. The test is performed before breakfast. A 5 ml. sample of blood is taken from a vein and allowed to clot. The patient is weighed and then injected with sterile 5% bromsulphthalein. Two mg. of dye are administered for every kg. of body weight. The injection should take about sixty seconds and care should be taken to avoid infiltration of the dye outside the vein. If any discolouration is noticed in the perivascular tissues the test should be abandoned. Exactly five minutes and thirty minutes after the injection, 5 ml. samples of blood are withdrawn from the other arm and allowed to clot. The specimens are centrifuged and the serum placed in tubes of standard bore similar to those employed for the standard dye solutions. An arbitrary 100% standard consists of 4 mg. of bromsulphthalein dissolved in 100 ml. water containing 0·25 ml. 10% sodium hydroxide. From this other standards, varying from 5 to 95% are prepared, by dilution with the mixture of water and sodium hydroxide solution. In comparing the serum with the standards, the former is treated with 2 drops of 10% sodium hydroxide to bring out the alkaline colour of the dye. The samples are compared in the usual way by holding a tube of water behind the coloured serum and the control serum behind the standard. Hæmolysed serum should be avoided in this test.

The test is positive, indicating liver damage, if any of the dye is retained in the blood at thirty minutes. The test lacks sensitivity. The *Serial Bromsulphthalein Test* as described by Macdonald (37 and 38) is more delicate. Blood specimens are obtained every five minutes for thirty minutes, by a method requiring only a single vein puncture, and the amount of dye injected is 5 mg. per kg. of body weight. Using 5 mg. per kg. of body weight, Mateer et al. (22) found that in normal individuals the blood should be cleared of the dye in the forty-five minute specimen ; false positive reactions may occur if a shorter time is taken for the standard. They consider that the distinction between a positive and negative test should lie in the complete disappearance of the dye from the blood and not in a percentage disappearance. The results obtained were very similar to those with the intravenous hippuric acid test.

Investigation of the Duodenal Contents (Lyon (39)). This has not proved to be of any practical value.

Conclusions. Tests depending on the pigmentary functions of the liver are used extensively and yield very reliable results. They do not always, however, indicate early changes. MacCormac and one of us (E. C. D.) (40) used Fouchet's test, and the urobilinogen and urobilin reactions for studying the hepatic changes in salvarsan treatment, and similar tests were used with Comyns Berkeley and Walker (41), for the study of liver function in eclampsia. In general hospital work they have been found very useful by most observers. It is difficult to estimate the exact clinical value of the van den Bergh reaction.

It would appear impossible to demonstrate fine hepatic lesions by metabolic changes, since the work of F. C. Mann (42) has shown that four-fifths of an animal's liver can be removed without causing any symptoms other than shock from the operation. If more of the liver is removed the animal has convulsions, as the result of hypoglycæmia, and can be instantly revived by the administration of dextrose solutions. This, after all, is only the experimental confirmation of what is frequently seen clinically. A liver may be riddled with secondary deposits of malignant growth, and yet the only symptom of deficiency in its function is jaundice. Both clinical and experimental evidence point to the remarkable powers of a badly damaged liver to carry on its normal functions, and, at the same time, to the impossibility of demonstrating fine hepatic lesions by means of tests depending on the metabolic functions of the liver.

Admittedly it is possible to state with relative surety, on the evidence supplied by these tests, that there is derangement of hepatic function, but, unfortunately, it is impossible to state more. Cirrhosis, carcinoma and fatty infiltration all produce more or less the same picture, and, since a diagnosis cannot be ventured on the results of hepatic efficiency tests, they must be considered somewhat of a failure from the medical aspect. Further, F. C. Mann (43) in 1942 says that he is not fully convinced that we are correct in speaking about liver function tests, and that the liver function varies, not only from day to day, but also from hour to hour.

Infective Hepatitis

Relationship to Catarrhal Jaundice. Epidemics of jaundice have been described for many years. In 1912 Cockayne (44) writing on sporadic and epidemic catarrhal jaundice, gave a historical review of epidemic catarrhal jaundice, one of the earliest references being to an outbreak in Minorca in 1745. In the British Isles epidemics have been recorded since the Birmingham outbreak in 1852. Each major war has witnessed similar epidemics ; they were recorded in the siege of Paris in 1870, in the Boer War, in Gallipoli during the 1914–18 war, and again in the 1939–45 war, chiefly in the Mediterranean theatre of operations.

Cockayne concluded that simple catarrhal jaundice, usually ascribed to gastro-duodenal catarrh, is, except in a comparatively small number of cases, the sporadic or endemic form of epidemic catarrhal jaundice. He thought that the infection was air-borne, that the incubation period was short, about five days, and that there was often a preliminary stage of naso-pharyngeal catarrh. Cockayne further considered that the liver was probably infected through the blood stream, and that inflammation may spread down the bile duct giving rise to catarrhal changes with obstruction. This corresponds very closely with the view now held by many that catarrhal jaundice is a sporadic form of infective hepatitis, and that in all cases the liver is affected by a blood-borne icterogenic agent, probably a virus.

The evidence, however, still exists that isolated cases of catarrhal jaundice are due to gastro-duodenal or bile duct catarrh, and we should not readily accept *ex-cathedra* statements to the contrary. Virchow (45) in 1865 supported the view that in catarrhal jaundice there is a state of duodenal catarrh involving the ampulla of Vater, with plugging of the orifice of the common bile duct with mucus. In 1908 Eppinger (46) described the post-mortem findings in a girl nineteen years of age who committed suicide by throwing herself out of a third floor window during an attack of jaundice associated with gastro-enteritis. The liver was enlarged and icteric and showed typical changes of mechanical jaundice. The common bile duct contained dark coloured and very viscous bile. The mucous membrane of the stomach showed hæmorrhages. The walls of the duodenum were catarrhal, and its contents

colourless. The papilla of Vater was prominent. Bile could not be forced through the common bile duct by strong pressure on the distended gall-bladder. Eppinger thought the obstruction was due to swollen lymphoid tissue near the ampulla of Vater and also at the junction of the cystic duct with the common bile duct.

On clinical grounds also certain authorities consider that catarrhal jaundice differs from infective hepatitis, for in catarrhal jaundice prodromal symptoms, apart from depression, are slight, the patient first taking to his bed when the jaundice is manifest. In infective hepatitis, on the other hand, the patient is ill, feverish, and confined to bed for several days before the jaundice appears.

More recently, Dible, McMichael and Sherlock (47), by aspiration biopsy observations on the liver, have found changes in sporadic cases of jaundice identical with those met with in infective hepatitis. Further, Van Rooyen and Gordon (48) by duodenal intubation studies in 11 cases of infective hepatitis found no evidence of duodeno-biliary catarrh. These experiments offer no proof that catarrhal jaundice, due to primary extra-hepatic catarrhal obstruction of the bile ducts, is a myth, as some would have us believe. All they show is that the cases investigated, which were assumed to be representative of catarrhal jaundice, were in reality examples of inflammatory hepatitis. It seems probable therefore that we should not be wise in abandoning Virchow's concept of catarrhal jaundice, and that, as Boyd (49) writes, "the basis of catarrhal jaundice may be (1) catarrhal obstruction of the opening of the bile duct, (2) toxic necrosis of the liver, and (3) cholangitis. Providing these lesions are transient, the jaundice will be catarrhal in type."

Epidemiology. Attention was again drawn to outbreaks of jaundice in England by Pickles (50) in 1930. He gave an account of 250 cases of epidemic jaundice in Wensleydale. The characteristic features were a pre-icteric phase, lasting from four to ten days, during which the patient suffered from nausea, vomiting, and pains in the back and limbs. This was followed by an icteric stage of about fourteen days. Pickles considered that the incubation period varied from twenty-six to thirty-five days, that the period of infectivity was short, and that the method of spread was respiratory, probably by droplet infection. In 1942 Newman (51) described an outbreak in the Lavant valley, in Sussex, but he did

not hold that the droplet theory of infection was entirely satisfactory. Fraser (52) in 1931 had brought forward evidence that the disease might be spread by water, and further evidence in favour of this view will be referred to later. Cookson (53) in 1944 reported an epidemic of 246 cases in Gloucestershire, chiefly amongst children attending elementary schools, or living in nurseries. He rejected the idea that the infection was water-borne. During the 1939–45 war, outbreaks of infective hepatitis occurred, especially amongst the troops in Syria, North Africa and Italy. An interesting account of over 1,000 cases of infective hepatitis amongst American troops in the Mediterranean area was given by Barker, Capps and Allen (54) in 1945. The epidemic occurred between February and November, 1944.

Ætiology. It has not been found possible to transmit the disease to animals, although Andersen (55) and Hallgren (56) reported the successful transmission of infective hepatitis to the pig, but other observers have failed to repeat the experiment. It is usually thought that the icterogenic agent is a virus. Hallgren (56) in 1940 published an account of epidemic hepatitis in the county of Västerbotten in Northern Sweden. In an outbreak at the Hällnäs Sanatorium evidence was obtained that the infection was water-borne, a corroded sewer having contaminated the well from which the drinking water was obtained. Thirty-four days after the infected well had been excluded, the epidemic came to an end. No new cases developed amongst 188 fresh patients who arrived after the exclusion of the well, although they were in contact with large numbers of sufferers from infective hepatitis. It was found that 32% of those exposed to infection were susceptible. A pig was given water from the polluted well and subsequently killed. Microscopical examination revealed necrotic and inflammatory liver changes resembling those of infective hepatitis, whereas nine control pigs showed normal livers. Cameron (57) injected intramuscularly six volunteers with 1 to 2 ml. of whole blood or serum obtained from patients suffering from infective hepatitis in Palestine. One man developed jaundice in a month, one within two months, and the other four within six months. As there was infective hepatitis in the district it was not possible to say that all the cases were due to the inoculations. Sheehan (58) in 1944 suggested that the icterogenic agent

was conveyed by biting insects, but this view has not been supported by other workers.

Neefe and Stokes (59) recorded an outbreak of infective hepatitis in a summer camp for boys and girls in the Pocono mountains, near Philadelphia. Out of a population of 573 there were 350 cases. It was shown that the water of the girls' well was contaminated by fæces from cesspools. Healthy volunteers were then inoculated with serum, naso-pharyngeal washings, urine, fæces and the infected water. It was shown that the icterogenic agent was present in the serum, fæces and infected water. The serum produced the disease in the human volunteers only when given orally, and not when injected parenterally. The incubation period was similar to that occurring in the epidemics. The agent in the fæces passed through a Seitz filter. No evidence was obtained of the transmission of the infection by biting insects. It was concluded that it was improbable that the infection was transmitted to the majority of persons by air, fomites, food, milk or directly from infected persons. Havens et al. (60) in 1945 also showed that the icterogenic agent is present in fæces, and that the disease can be reproduced in man by feeding such material in gelatin capsules or by spraying it into the nasal and pharyngeal passages. Further, jaundice can be produced by feeding serum from cases of infective hepatitis. The average incubation period was twenty-eight days. The ætiological agent, probably a virus, is in the blood stream before the onset of, and during the jaundice. It is present in the fæces during the acute stage of the disease, and it probably also occurs in the nasal washings. It is filtrable, and markedly heat-stable, resisting a temperature of 56° C. for half an hour. Infection can probably be spread by flies contaminated by infected excreta, as is the case with typhoid fever. Findlay and Willcox (61) also showed that the oral ingestion of fæces or urine, derived from spontaneous cases of infective hepatitis will cause typical icteric and sub-icteric cases of the disease. Seventeen volunteers were given to drink 30 to 50 ml. of urine mixed with an equal quantity of milk, and 5 developed hepatitis. Further, 3 g. of fæces were ground up in 250 ml. of milk and 50 ml. were given to each volunteer to drink on an empty stomach. Positive results were obtained in 11 out of 47 cases.

Alcohol appears to play a part predisposing to infective hepatitis. In the Services the disease had a higher incidence amongst officers than amongst other ranks, possibly due to the greater amount of spirits consumed by the former. Hartfall (62) gives examples of the deleterious effects of alcohol "upon an individual who is incubating the disease, in provoking an overt attack, or in worsening or prolonging an attack otherwise mild, and in causing a relapse or second delayed attack of jaundice."

Pathology. The disease is probably a systemic infection with a predilection for the liver. Dible, McMichael and Sherlock (47) studied the pathology of acute hepatitis in 56 cases by aspiration biopsy, and found no histological criteria for differentiating the lesions resulting from epidemic hepatitis, arsenotherapy and serum inoculations. The cells of the hepatic lobules show evidence of necrosis and autolysis. In the areas around the central veins there is rarefaction due to the disappearance of liver cells. Small mononuclear cells of the histiocytic class, with some polymorphonuclears and eosinophils, tend to accumulate in the portal zones. These infiltrations may spread around the periphery of the lobules. There is enlargement and hyperplasia of the sinusoidal "endothelial" cells. There does not appear to be any fatty change in the cells. In over a third of the cases proliferation of the bile ducts was seen in the portal tracts. It appears that in the early stages the lesion is chiefly limited to the portal zone, with little destruction of the liver lobule. The liver inflammation probably begins with the prodromal symptoms. Bile thrombi may be seen in the intralobular bile canaliculi. In some cases a permanent cirrhosis develops, in others there is a mild residual fibrosis, which may ultimately completely resolve.

Technique of Liver Puncture. Sherlock (63) recommends that a vitamin K preparation should be given for three days previously. If urobilin is present in the urine, indicating that the jaundice is non-obstructive, two 5 mg. tablets of Kapilon are taken by mouth three times a day, but if the jaundice is obstructive 5 mg. of Kapilon should be injected intramuscularly daily. The puncture is made in the ninth or tenth right intercostal space in the mid or anterior axillary line, with the patient lying supine, the right arm being placed behind the head, and a pillow wedged under the left side, to tilt the body slightly to the right. Ten ml. of

2% procaine are used to anæsthetise the tissues including the pleura, the diaphragm, the peritoneum and the liver capsule. A cannula 15 cm. long and 1 mm. in bore, fitted with a handled trocar is passed through the skin, and the patient is told to take a deep breath in, let it out, and then hold the breath. The trocar and cannula are now pushed through the diaphragm into the right lobe of the liver, and the trocar withdrawn when the instrument is half an inch inside the liver substance. The cannula is then pushed in a further 2 inches, when a 20 ml. "Record" syringe is attached to the cannula and suction applied and maintained while the cannula is withdrawn. The puncture should be performed rapidly, and it is dangerous for the patient to breathe during the operation, as this may cause a tear in the liver. The puncture wound is sealed with collodion. The fragment of liver is usually found in the barrel of the syringe, occasionally it remains in the cannula. It may be necessary to give an injection of morphine subsequently to relieve pain, and a careful watch must be maintained for hæmorrhage during the next twenty-four hours. The pulse should be charted hourly, and if there are signs of hæmorrhage a cross-matched blood transfusion should be given.

Clinical Findings. The mode of onset, course and severity of the illness are variable. Four clinical modes of onset have been recognised: (1) Insidious, with anorexia and gastro-intestinal symptoms; there is a low grade pyrexia, usually under 100° F., and jaundice appears after three or four days. (2) Febrile, the temperature rising suddenly at the onset to 101° to 103° F. Jaundice occurs two to four days later. (3) Ambulatory, the patient continuing with his work until he is noticed to be jaundiced. (4) Hepatitis without any manifest jaundice. Hartfall (62) in Malta observed that in 53% the onset was insidious, and in 30% it conformed to the febrile type. Barker, Capps and Allen (54) in the Mediterranean theatre found that amongst American troops in over 80% of cases there was an acute febrile onset, the temperature rising to 100° to 103° F., associated with malaise, headache, lassitude, anorexia, nausea and vomiting. The patient complained of chilly sensations, rarely of rigors, with abdominal discomfort and pains and tenderness in the epigastrium and right hypochondrium. Urticaria, morbilliform rashes and arthralgia were

occasionally met with. After three or four days the temperature fell to normal, the liver gradually enlarging and becoming tender. The spleen was rarely palpable. Constipation and diarrhœa were equally common. This pre-icteric stage was followed five to ten days later by the icteric stage, the temperature again rising. Nausea and abdominal discomfort were more marked, the jaundice lasting from 2 to 4 weeks or in some cases for three months or even longer. In the average case recovery was complete in from six to eight weeks. In this outbreak the stools were rarely clay-coloured, in other epidemics pasty stools were the rule. Cameron (57) in an outbreak in Palestine amongst the troops found the liver enlarged in 97 out of 170 patients, and the spleen enlarged in forty-six cases. Clay-coloured stools were found in half the cases, in 12 cases the stools were normal in colour, in the remainder they were light coloured. In fatal cases, which are rare, the jaundice becomes more intense, hæmorrhages may be seen in the skin, or there may be pulmonary or intestinal bleeding, and, in some cases, ascites develops. The patient becomes irrational and dies in coma.

Nervous complications are uncommon, but meningitis, encephalitis, polyneuritis, myelitis and upper motor lesions of the pyramidal and striatal type have been described by Lescher (64), Byrne and Taylor (65), and Stokes, Owen and Holmes (66). In none of these cases could it be proved that the nervous lesions were in reality due to the icterogenic agent, in all they might have been due to intercurrent causes. The death rate from infective hepatitis is very low, but in the Mediterranean area it was higher in native than in white troops. Relapses are rare, but we have seen one patient who had three relapses and died suddenly from hæmorrhage due to ruptured œsophageal varices.

Laboratory Findings. *The Blood.* The white cell count is normal or there is a slight leucopenia. A leucocytosis would indicate that the diagnosis is probably incorrect, and leucocytosis is a feature of Weil's disease. The sedimentation rate of the red cells was found by Miles (67) to be usually normal during the icteric stage of the disease, but often raised in the pre-icteric period and during convalescence. This author suggests that an excess of bile salts in the blood is responsible for the low sedimentation rate during the active phase of infective hepatitis. Barker,

Capps and Allen (54) also reported that the sedimentation rate is usually normal in the presence of jaundice. Evans (68) found that the sedimentation rate may remain raised for some weeks after an attack. Hoagland and Shank (69) in a series of 163 cases found that the sedimentation rate was normal or only slightly raised, during the first days of the disease, but frequently raised to values of 25 to 55 mm. per hour during convalescence and gradually falling to normal late in the period of recovery. The serum bilirubin is generally normal during the pre-icteric stage, rising later to levels of 10 mg./100 ml. or higher, according to the intensity of the jaundice. The Van den Bergh test usually gives a positive direct and indirect reaction falling to normal after about forty days or less.

The Urine. During the pre-icteric stage urobilinogen may, or may not be found in the urine ; with the onset of overt jaundice bilirubinuria occurs.

Liver Function Tests. Pollock (70) using the intravenous hippuric acid test found that the synthesis was to a large extent correlated with the severity of the attack and the stage of the disease, but varied only partly. and indirectly with the serum bilirubin concentration. The hippuric acid synthesis was noted to be improving while the bilirubin level was still rising, and it appeared that liver damage was probably present in the pre-icteric period. Gordon (71) employing the oral hippuric acid test found evidence of impaired liver function in all of 14 cases examined during the first three weeks of jaundice. Barker, Capps and Allen (54) observed that the sulphbromophthalein test was positive in all cases examined during the first five weeks of the illness, and the colloidal gold test was usually positive during the icteric stage. Hallgren (56) found that the galactose tolerance test was positive in 12% of cases. Britton (34), using his special technique for the Takata-Ara test, found a positive reaction beginning within two to three days of the onset of the jaundice, the strength of the reaction following closely the severity of the liver damage and the course of recovery.

Differential Diagnosis. During the pre-icteric stage infective hepatitis may be mistaken for influenza, gastro-enteritis, sand-fly fever, malaria, meningitis, acute appendicitis, enterica group infections, or an alcoholic " hang-over."

Treatment. *Prophylactic.* Gamma globulin, obtained from large pools of adult human serum, was found by Stokes and Neefe (72) to be a potent agent in the prevention or attenuation of infective hepatitis, if injected during the incubation period. Gellis et al. (73) confirmed this view in two epidemics in the Mediterranean theatre of operation. This beneficial effect is possibly due to neutralising antibodies present in the gamma globulin. Over 1,700 men were inoculated intramuscularly with 10 ml. of globulin, and more than 10,000 men served as controls. The inoculated belonged to units in which the disease was already rife. The incidence of hepatitis in those injected was 0·6%, but of the uninoculated 3% became infected.

Curative. Complete rest in bed from the onset appears to shorten the course of the disease in the majority of cases. It is usually advised that the diet should be high in protein (200 g.) and in carbohydrate (400 g.) and low in fat (40 g.). Darmady (74), on the other hand, considered that there is no difference in the results of treatment whether a high or a low protein diet is given. A light diet with plenty of milk, from which the top layer of cream has been removed, is usually perfectly suitable. During the acute stage the patient should take fluids up to 5 pints a day. If this amount cannot be taken by mouth, 10% dextrose in water should be given intravenously, or if vomiting persists 5% dextrose in saline should be transfused to make up the deficiency. If there are hæmorrhages or if the plasma prothrombin content is low, vitamin K should be injected intramuscularly in doses of 5 mg. daily, and for serious hæmorrhage a blood transfusion should be given. The patient should take no alcohol during the disease or for a year subsequently. Methionine, an essential amino-acid, and a lipotropic factor which promotes the transfer of fat from the liver to the storage depots, has been recommended as an aid to treatment, but Wilson, Pollock and Harris (75) giving 5 g. of methionine daily by mouth to alternate patients in a series of 100 cases of infective hepatitis found that the methionine, as judged by clinical and biochemical criteria, had no significant effect upon the severity or duration of the disease, or the incidence of relapses. Similar results were obtained by Higgins et al. (76). Finally, the patient should not be allowed to get up until the temperature is normal, the liver has returned to its accustomed

size and is no longer tender, there are no symptoms such as lassitude and nausea, and the serum bilirubin has been normal for a week.

Homologous Serum Jaundice

It is believed that homologous serum jaundice results from the introduction of an icterogenic substance into man by the injection of human blood, serum, or plasma. The amount of serum injected may be very small, as little as 0·05 ml.

As far back as 1885 Lurman (77) reported an outbreak of jaundice following vaccination against small-pox with "glycerinated humanised" lymph. Out of 1,289 workers so vaccinated, in a ship-building yard at Bremen 191 developed jaundice several weeks later, whereas 500 others, vaccinated with a different batch of lymph, were unaffected.

We now know that homologous serum jaundice may arise in a variety of ways, of which the following are the most important :— By the injection of yellow fever vaccine prepared with human serum ; as the result of prophylactic inoculation with measles convalescent serum or mumps convalescent plasma ; and following transfusion with whole blood, plasma or serum. It may also result from the use of syringes contaminated with infected blood, and employed either for intravenous, intramuscular or subcutaneous injections, or for removing blood by venepuncture. This latter group includes post-arsenical jaundice occurring in venereal disease clinics, jaundice following the injection of penicillin or pentothal, and occasional outbreaks of jaundice in clinics for the treatment of diabetes mellitus and arthritis, or in sanatoria. These varieties will now be considered in more detail.

Jaundice following Yellow Fever Vaccination. Findlay and MacCallum (78) in 1937, and subsequently, drew attention to the occurrence of jaundice following immunisation against yellow fever with a vaccine consisting of yellow fever virus suspended in human serum. They observed 89 cases among 3,100 persons immunised over a period of five years, the latent period varying between thirty-six days and seven months, and averaging between two and three months. They showed that the occurrence of the jaundice was not directly due to an infection with the yellow fever virus, and considered that there was presumptive evidence that

the post-inoculation jaundice was identical with infective hepatic jaundice. Findlay, MacCallum and Murgatroyd (79) later showed that the icterogenic agent bore no relationship to the strain of yellow fever virus used, but that experiments suggested the presence of an extraneous virus in the human serum used in the preparation of the vaccine. In 1939 Fox, Manso, Penna and Para (80) published an account of an outbreak of inoculation jaundice in Brazil, after over a million people had been inoculated there against yellow fever. Out of a group of 304 persons inoculated with one batch of vaccine, 27% developed jaundice four to five months later, one case proving fatal. The occurrence between January 1st and July 1st in 1942 of 28,585 cases of jaundice, 62 of which proved fatal, out of between two and two and a half million American troops inoculated against yellow fever, was commented on in Editorials in the American medical press (81). Widespread attention was directed to this variety of jaundice, which was again shown to be due to the presence of an icterogenic agent in the serum used as a vehicle in the preparation of the vaccine. The latent period in this series varied between twelve and eighteen weeks. In 1944 Findlay, Martin and Mitchell (82) reported on a series of 689 cases of jaundice following yellow fever vaccine injections given during 1942, the average latent period being 101·5 days. There was one fatal case. Human serum is no longer used in the preparation of the vaccine and this type of jaundice has now ceased.

Jaundice following Measles Convalescent Serum. In the Annual Report of the Chief Medical Officer of Health for 1937 (83) there was a brief account of the occurrence of 37 cases of jaundice, 7 of which proved fatal, 16 to 100 days after the injection of measles convalescent serum from two small batches into between 82 and 109 recipients. Propert (84) in 1938 drew attention to the cases of 7 children inoculated with convalescent measles serum of the same batch, who all developed jaundice 78 to 83 days later, 3 cases proving fatal. The pooled serum used was the same as that referred to in the Ministry of Health report. A fuller account was reconstructed from contemporaneous records and published in a Memorandum from the Ministry of Health in 1943 (85), as the matter was now considered to be one of major importance. It was thought that the jaundice was due to the injection of human

blood products, and that probably further cases would occur, particularly after transfusion, and such indeed has proved to be the case.

Jaundice following Mumps Convalescent Plasma. Beeson, Chesney and McFarlan (86) in 1944 reported the occurrence of 101 cases of jaundice following the inoculation of 266 men with mumps convalescent plasma. The onset was in the majority of cases between the fifty-ninth and ninety-fourth day after inoculation. The jaundice was considered to be due to a hepatoxic agent in two batches of plasma.

Jaundice following Transfusion. Cases of jaundice following at an interval of about three months after blood transfusions were recognised in England in 1942. Morgan and Williamson (87) reported that 9 patients who were treated with large transfusions of plasma or serum, 7 suffering from intermittent claudication, 1 from diabetes mellitus with gangrene of the toes, and 1 from calcinosis circumscripta, developed jaundice seven to ten weeks later. It was not possible to trace the icterogenic agent to definite batches of serum or plasma. The authors stated that the jaundice might be due to a virus or to some toxic chemical substance, such as altered plasma protein, but there was no evidence to favour one or other of these hypotheses. Beeson (88) in America, in 1943, reported 7 cases of jaundice occurring one to four months after the transfusion of whole blood or plasma, and concluded that isolated cases may not infrequently be met with. Steiner (89) also recorded 5 cases of jaundice following transfusions with whole blood or human plasma, given to soldiers when they were wounded two to three months previously. There was no proof in any case that an icterogenic agent was transferred by the transfusion, the evidence being purely presumptive.

We now pass on to the other type of serum jaundice, in which it is believed that the causative agent is transmitted by contaminated syringes.

Post-arsenical Jaundice. Bigger (90), in 1943, drew attention to the increased incidence of post-arsenical jaundice in venereal disease clinics. In one of these the case incidence rose from 4·2% in 1941 to 16·5% in 1942. Beattie and Marshall (91) found an incidence of over 50% of post-arsenical jaundice at a clinic in 1942 and 1943, the jaundice usually developing between the

ninety-first and one hundred and sixty-sixth day from the first injection. Bigger (90) showed that the icterogenic agent was probably transmitted by syringes contaminated by a trace of blood from an infective patient, and hence the term "syringe jaundice" or "syringe-transmitted hepatitis" came into use. When an intravenous injection is given a minute amount of blood enters the syringe after the needle is put into the vein, and Bigger, using in his experiments citrated blood contaminated with staphylococci, showed that the methods in use at the clinics for sterilising the syringes, such as soaking in 1/1,000 biniodide of mercury, and washing in four changes of water, were not adequate to remove minute quantities of infected material. He recommended that the syringes should be boiled between each patient. It was subsequently proposed by the Medical Research Council Syringe Sterilisation Committee that all-glass syringes should be sterilised by dry heat at 160° C. for an hour. Salaman, King, Williams and Nicol (92) suggested that the increase in post-arsenical jaundice during these years might be due to a variety of causes, such as a shortage of syringes and an increase in the number of patients, with a consequent diminution of the time the syringes could be soaked in disinfectants, and also to an increased incidence of infective hepatitis, if we accept the theory that the blood from patients incubating infective hepatitis is the source of the icterogenic agent. There is a further possibility, the low protein content of the war-time diet or increased alcoholic consumption may have diminished the resistance of the liver to the icterogenic agent. Sheehan (58) observed that some orderlies at a venereal disease clinic, who helped in the injections and had their hands contaminated with blood from the patients, developed jaundice, presumably through scratches in the skin. Beattie and Marshall (91) tried to lower the incidence of post-arsenical jaundice at a clinic by giving the patients a casein digest, a casein digest with cystine, or methionine. These measures had no effect on the over-all incidence of liver damage and jaundice, but tended to shift its incidence to later in the course of treatment, and to render the jaundice less severe.

Jaundice following the Injection of Penthothal and Penicillin. Darmady and Hardwick (93) observed 6 cases of jaundice following the intravenous injection of pentothal between 31 and 151 days

previously, and 4 cases 64 to 113 days after the intramuscular injection of penicillin for the treatment of gonorrhœa or syphilis. They suggested that here again the jaundice resulted from the use of " dirty " syringes. They further considered that some cases of transfusion hepatitis may be due to the same cause and not to the blood injected, pentothal or penicillin having been given at the time of the transfusion. Howells and Kerr (94) treated 47 patients suffering from jaundice, who had previously received injections of penicillin, the average latent period being 97 days. Of these cases, 41 had been given penicillin for the treatment of gonorrhœa or syphilis. They concluded " that penicillin is not the direct cause of the hepatitis, which may be due to some icterogenic agent—*e.g.*, a virus—transmitted by faulty injection technique."

Jaundice following the Withdrawal of Blood. Graham (95) was puzzled by the occurrence of 28 cases of jaundice developing during a period of two and a half years in patients suffering from diabetes mellitus, treated at hospital, none being noted in private patients during the same period. He wrote, " I have come to the conclusion that the jaundice must be due to an infective agent passed from patient to patient at hospital." He suspected that the infection was conveyed by the syringes. This might have occurred when blood was removed for sugar estimations, or when insulin was injected. Droller (96) described an outbreak of jaundice in a diabetes clinic in which 62 patients were affected during a period of two years, 4 of the cases proving fatal. Blood was collected for sugar estimations in syringes which were not boiled, and it was thought that small amounts of infective material might have contaminated the needles. Damodaran and Hartfall (97) observed cases of jaundice in a clinic at which patients suffering from arthritis were treated with gold injections. They do not state whether blood was removed for sedimentation readings. In 1935 there were no cases of jaundice, but later, when there was a sparse outbreak of infective hepatitis, up to 9·4% of the patients at the clinic developed jaundice. They did not recognise infected syringes as a possible cause of the jaundice. Sheehan (58) in the same year reported cases of jaundice occurring in a sanatorium. It was noted that a patient who developed jaundice had had a vein puncture for the estimation of the sedimentation rate of the red cells about three months earlier, and

on the same day as another patient in the ward who was destined to develop jaundice a month or two later, the incubation period being therefore about twelve weeks. In all these cases it is probable that the syringe was contaminated by a small quantity of blood containing the icterogenic agent, and that, as was shown by Mendelssohn and Witts (98), on removing the needle from the vein, if the tourniquet is released before the needle is withdrawn, a small quantity of blood is sucked back from the needle and the syringe into the vein. If the syringe, therefore, is not sterile, the patient is exposed to infection.

The Nature of the Icterogenic Agent. It has been suggested that infective hepatitis and homologous serum jaundice are both due to the same agent, a virus, but there is no proof of this view. There is, indeed, evidence that the two conditions are dissimilar and result from different causes.

The icterogenic agent has been found to behave in many respects like a virus; it passes through Seitz filter-pads, and resists freezing and drying for long periods, but it has never been transmitted to the lower animals. Findlay and Martin (99) in 1943 instilled nasal washings from patients suffering from jaundice following yellow fever inoculation intranasally into volunteers. In three out of four instances jaundice resulted at intervals of 28, 30 and 56 days. They suggested that infective hepatitis and homologous serum jaundice are due to the same virus, the difference in the incubation period being due to the infection occurring intranasally in infective hepatitis, and by subcutaneous injection in homologous serum jaundice. Findlay, Martin and Mitchell (82) in 1944 failed to produce jaundice in volunteers by the intranasal instillation of whole blood or the subcutaneous injection of whole blood from patients suffering from post-inoculation jaundice. MacCallum and Bauer (100) showed that serum from a presumed case of homologous serum jaundice was icterogenic when inoculated subcutaneously or intramuscularly into a volunteer, on the seventh day after the onset of jaundice, but not so 59 and 134 days later. A further point in favour of the view that infective hepatitis and serum jaundice are manifestations of the same disease is afforded by the work of Dible, McMichael and Sherlock (47). They examined microscopically specimens obtained by liver puncture, and could detect no

difference in the appearance of the liver in infective heptatitis, post-arsenical jaundice, and serum jaundice. On the other hand there is evidence that infective hepatitis and serum jaundice are not due to the same virus. Homologous serum jaundice does not afford immunity against infective hepatitis. Sufferers from serum jaundice have not been proved to infect contacts, although there is some evidence that this may very occasionally happen. The incubation period of the two diseases is very different, about 30 days for infective hepatitis, and about 100 days for serum jaundice. Some authorities have also pointed out differences in the clinical features, especially the occurrence of erythema multiforme, stiff joints, splenomegaly, and the usually apyrexial course in serum jaundice. MacCallum (101) has summed up the position as follows : " There are many debatable points in favour of and against the identity of infective hepatitis and homologous serum jaundice, but there is no conclusive evidence one way or the other. It is essential to find a satisfactory susceptible experimental animal before much further knowledge can be obtained."

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CHAPTER VI

THE STOMACH

X-ray Examination

Examination of the stomach and of the duodenum by means of the X-rays after the ingestion of an opaque meal is a useful adjunct in the differential diagnosis of ulcers of these organs.

Investigations should be made both under the screen and with films. The opaque meal used in the screen examination usually consists of 3 oz. of barium sulphate in half a pint of milk. By this means the shape of the stomach and its filling properties can be investigated.

The motor power and rate of stomach emptying are better studied with a more solid meal, such as porridge or bread and milk containing barium sulphate. There should be no residue of such a meal in the stomach after six hours.

Persistent deformity in the outline of the stomach or duodenum affords some radiological indication of ulcer. Thus in gastric ulcer the barium may be seen filling a niche in the wall of the stomach, or there may be a definite organic hour-glass appearance. In the duodenum a persistent alteration in the shape of the duodenal cap may be seen. The X-ray examination will also reveal pyloric obstruction.

Variations in tone or in the position of the stomach are not diagnostic of ulceration. Subsequent examination after the administration of belladonna is of value in distinguishing between constriction of the stomach due to spasm and that due to cicatrisation.

Study of the relief pattern of the gastric and duodenal mucous membrane has been stimulated by the work of Berg (1) using the " aimed exposure with dosed compression " technique. One or two mouthfuls of a suspension of barium sulphate in water are swallowed, and a thin layer is thus spread over the gastric mucous membrane. With the patient under the screen, compression, in varying degrees, is exerted by a small lead-lined cylindrical applicator (" dosed compression "). If a permanent

record is required the film must be exposed within a fraction of a second of seeing the object on the screen (" aimed exposure "). In this way the radiological appearances of normal or abnormal mucosal folds can be demonstrated, and, by suitable rotation of the patient, an ulcer niche may become apparent either *en face* or *en profile*. Direct rather than indirect radiological evidence of an ulcer may thus be obtained. Cordiner and Calthrop (2) have published details of this method as applied to the radiography of the duodenal cap, and they emphasise its importance.

Gastroscopy

The introduction of the flexible gastroscope in 1932 by Schindler and Wolf and of the Hermon Taylor (3) model with controllable flexibility in 1941 has further advanced the study of gastric diseases. A biopsy attachment to the Wolf-Schindler instrument has been developed in America. The patient should be examined fasting, using 2% Anethaine as a local anæsthetic. A preliminary subcutaneous injection of morphin. sulph. gr. $\frac{1}{4}$ and atropin. sulph. gr. 1/120 is given three-quarters of an hour before instrumentation, and a gr. $1\frac{1}{2}$ Anethaine pastille is sucked thirty minutes later. If the pharynx is still sensitive, 3 ml. of 2% Anethaine are used in addition as a gargle. Overdosage may result in toxic symptoms. Rodgers (4) gives the following indications for gastroscopy : When the skiagram has shown some abnormality but no definite diagnosis has been made, or when, a diagnosis having been made, some additional information is required. It is helpful in cases of dyspepsia in which the skiagram has revealed no abnormality. In certain cases of anæmia its use is of interest, although of little practical value. There are also contra-indications, which include local conditions such as trismus, œsophageal stenosis, enlarged mediastinal glands, an aneurysm or severe curvature of the spine. A large carcinoma near the cardiac orifice may render the examination impossible, and it should not be performed within a few days of a severe hæmatemesis. The abolition of the " blind area " in the stomach has been largely effected by the use of the Hermon Taylor gastroscope. In some cases of doubtful X-ray diagnosis of carcinoma a definite diagnosis, either positive or negative, may be made by gastroscopy.

On the other hand, a lesion which has been seen through the gastroscope and diagnosed as an ulcer, may be proved by operation to be a carcinoma some weeks later. Like most methods of investigation, gastroscopy is not infallible. Gastric ulcers may frequently be visualised and be seen to heal under medical treatment. In some cases an ulcer may be seen which is not demonstrable radiologically and the converse is also true. Lintott (5) found that gastroscopic healing of gastric ulcers did not occur until one to several weeks after the disappearance of all X-ray deformity. A gastro-jejunal stoma is not always easy to see.

It is in the diagnosis of gastritis that gastroscopy appears most useful as has been emphasised by Bank and Renshaw (6) and by Freeman (7). During the active stages of inflammation the mucous membrane shows hyperæmia, œdema, exudation, hæmorrhages and superficial ulceration. The results of inflammation are granularity of the mucous membrane, narrowing and distortion of the folds, and atrophy. Gill et al. (8) examining 217 service cases of dyspepsia gastroscopically found gastritis as an isolated lesion in 32%, and gastritis was present as an associated lesion in 80% of cases of gastric ulcer, and in 60% of cases of pyloric and duodenal ulcer. Berry (9) studied gastroscopically 100 alcohol addicts, and found that only 35% had unequivocal alcoholic gastritis.

In pernicious and simple achlorhydric anæmias the mucous membrane is thin, pale and as described by Hartfall (10) the most characteristic feature in pernicious anæmia "is the presence of branching submucosal vessels which impart a bluish network to the thin, pale, wash-leather background." The pallor is probably due to anæmia and is much less marked after adequate treatment, but the mucous membrane remains thin and does not appear to regenerate. The changes in the simple achlorhydric anæmias are very similar, but the atrophy is less severe. There does not appear to be any evidence of a preceding gastritis.

Principles of Treatment of Gastric and Duodenal Ulcers

Various modifications in the treatment of ulcers of the stomach and duodenum have been introduced during recent years. From

the practical point of view the subject can be considered under the following headings :—

The treatment of hæmatemesis due to gastric or duodenal ulceration.

The medical treatment of ulcers not at the time complicated by hæmorrhage.

The indications for surgical treatment of gastric and duodenal ulcers, not complicated by hæmorrhage.

Hæmatemesis. It is probable that between 10% and 30% of all peptic ulcers bleed, but the hæmorrhage is not necessarily severe, and the average death rate from hæmorrhage has been variously estimated as follows : Cullinan and Price (11) give a mortality of 10% in a series of 105 cases of the hospital class, 4 of which were treated surgically, all dying. Hurst and Ryle (12) in 677 cases of the private practice class had a mortality of 1·5%. Three of their cases were treated surgically and all died. Meulengracht (13) records a mortality of 1·3% in 368 cases of the hospital class treated by early feeding.

Discussions at the Royal Society of Medicine, London, in 1924, and again in 1934 (14) emphasised that the immediate treatment of acute hæmatemesis due to gastric or duodenal ulcer is almost always medical. It has usually been considered that the likelihood of death rises with the recurrence of hæmorrhage, a primary hæmorrhage not being so dangerous as a subsequent one. This view is not borne out by statistics from Seattle (U.S.A.). Thus Blackford and Allen (15) in 1942 recorded 120 deaths in men, and 31 deaths in women from bleeding peptic ulcer. Seventy-seven per cent. of all fatalities occurred during the first hæmorrhage, and only 8 deaths occurred in patients under forty-five years old. In patients under forty years of age fatalities are almost unknown. The surgical view-point will be referred to later.

Medical Treatment of Severe Hæmatemesis. It must be clearly understood that the type of patient for whom this treatment is required is so desperately ill that he is not able to take feeds by mouth. (*a*) The patient is put to bed, kept warm and very still. (*b*) Morphine sulphate gr. $\frac{1}{4}$ and atropine sulphate gr. $\frac{1}{100}$ are injected subcutaneously. This may be repeated up to a total of morphine gr. 1 in twenty-four hours. Willcox (16) has drawn attention to the danger of morphine in cases of hæmatemesis due

to cirrhosis of the liver, when the detoxicating power of the liver may be so diminished that even gr. $\frac{1}{4}$ may cause death; our experience supports this view. (*c*) Nothing is given by mouth for one to two days. Ice should not be sucked, but attention must be given to the toilet of the mouth, it being rinsed out from time to time with water. (*d*) An hourly pulse chart should be kept. (*e*) The blood pressure and hæmoglobin percentage should be determined every twelve to twenty-four hours. (*f*) The blood urea should be estimated, and, if raised, determinations should be made daily until it falls to normal. (*g*) The bowels should not be opened for four to seven days, an enema then being given. (*h*) Rectal salines of 6 to 8 oz. with dextrose 2% should be given every four hours, or a rectal drip may be used with a Murphy vulcanite rectal nozzle, which allows free escape of flatus backwards along the tube to the reservoir, as described by Marriott and Kekwick (17). Six to 8 pints of water, normal or isotonic saline (0·85% sodium chloride) or isotonic dextrose (5% dextrose) may be run in slowly every twenty-four hours, resting the bowel every other hour by interrupting the flow. (*i*) Blood transfusion is indicated, according to Witts (18), if the hæmoglobin is below 40%, the systolic blood pressure below 90 mm. Hg., the pulse rate over 140, or the blood urea over 100 mg. per 100 ml. The transfusion may be either a small one of 250 ml. repeated as required, or the drip method advocated by Marriott and Kekwick (19) may be used. The amount of blood given by either method should raise the hæmoglobin to about 80%. One pint of blood administered to an adult will increase the hæmoglobin by about 10%. By the drip method this 10% increase should be achieved every four hours. (*j*) The value of hæmostatics is open to question. Five or 10 ml. of "Stypven" containing 0·5 mg. or 1 mg. of Russell's viper venom may be given by mouth, or 5 ml. calcium gluconate (10%) intramuscularly once daily.

After periods of one to two days, and when the bleeding has been arrested, as judged by absence of hæmatemesis and the general condition of the patient, 4 oz. of half-strength normal saline (0·42% sodium chloride) should be given by mouth every four hours for twelve hours. Milk feeds may now be begun, first with milk 1 oz., water 1 oz. and emulsio mag. oxid. m. 30, every two hours, and then milk 2 oz., every two hours and gradually

increasing the milk feeds to 5 oz. every two hours. Tincture of belladonna (m. 10–15) should be given before three feeds, and olive oil (½ fl. oz.) alternately before three other feeds. A teaspoonful of an alkaline powder (see p. 185) or of Aludrox should be given after five feeds, and a double dose at night. Ferri et ammon. citrat. gr. 30 should be given three times a day between the feeds. The subsequent medical treatment is as for the treatment of ulcer not complicated by hæmorrhage, and is detailed later.

Routine Medical Treatment of Hæmatemesis. In the majority of cases of hæmatemesis the patient can tolerate early feeding and does better with it. The stomach is not rested by starvation. The starvation treatment is now being generally abandoned except in the very severe cases, as mentioned above. With a turn of the wheel we are back where we were in 1904 when Lenhartz (20) introduced his diet for immediate feeding in hæmatemesis, which was described in earlier editions of this book. Meulengracht (13) has published figures showing a lower mortality rate than has been obtained by other methods of treatment, and he advocates a liberal dietary from the first day of bleeding. The routine he recommends is as follows: 6 *a.m.* Tea, white bread and butter. 9 *a.m.* Oatmeal with milk, white bread and butter. 1 *p.m.* A selection from: meat balls, timbale, broiled chops, fish balls, vegetable gratin, fish gratin, mashed potatoes, vegetable purée, vegetable soups, cream of vegetables, stewed apricots, apple sauce, gruel, rice and tapioca puddings. 3 *p.m.* Cocoa. 6 *p.m.* White bread and butter, sliced meats, cheese and tea. The patients are allowed as much as they like. In addition, one teaspoonful of the following powder is given: Sod. bicarb. gr. 30, mag. carb. lev. gr. 30, ext. hyoscyam. sicc. gr. 4, and ferri lact. gr. 5, t.i.d.

We have tried this method with satisfactory results in a few cases, but in others it has proved impracticable as the patient could not take the diet. We doubt whether the true Meulengracht diet is now ever employed in this country, the fact being that we can seldom stomach it. There are, indeed, numerous modifications thereof which masquerade under the name of Meulengracht and cause much confusion. Witts (18) recommends a diet which is more fluid than that of Meulengracht and contains no meat, but which conforms to the principles of feeding by mouth on the first

day of the hæmorrhage. This has proved of great value in the treatment of many cases of hæmatemesis. Thus Pickering (21) writing in 1942 says that during the last six years he has treated all his patients (over 40 in number) suffering from gastric and duodenal hæmorrhage by the diet recommended by Witts, with only one death, and he supports Witts' view that " the results of

DIETETIC TREATMENT OF GASTRO-DUODENAL HÆMORRHAGE ARRANGED FOR TWO-HOURLY FEEDING

Feeds by Day	Food	Day		
		1	2	3 and subsequent.
1	Whole milk (fresh or dried) . . oz.	5	5	5
	Patent barley or strained porridge .	Portion	Portion	Portion
2	1 egg beaten up in milk . . oz.	5	5	5
	Buttered rusks or cream crackers . .	–	1	2
3	Whole milk (fresh or dried) . . oz.	5	5	5
	Marmite to taste	–	–	–
	Barley sugar . . . . oz.	1	1	1
	Thin crustless white bread and butter slices	–	1	2
4	Strained orange or tomato juice oz.	1	1	1
	Vegetable purée	Portion	Portion	Portion
	Pudding	,,	,,	,,
	Cream . . . . . oz.	1	1	1
	Boiled or steamed fish . . .	–	–	Portion
5	1 egg beaten up in milk . . oz.	5	5	5
	Barley sugar . . . . oz.	1	1	1
	Buttered rusks or cream crackers . .	–	1	2
6	Whole milk (fresh or dried) . . oz.	5	5	5
	Fruit purée	Portion	Portion	Portion
	Pudding	,,	,,	,,
	Cream . . . . . oz.	–	1	1
	Thin crustless white bread and butter slices	–	1	2
7	1 egg beaten up in milk . . oz.	5	5	5
	Black treacle or barley sugar . oz.	1	1	1
	Buttered rusks or cream crackers .	–	1	2
8	Whole milk (fresh or dried) . . oz.	5	5	5
	Fruit purée	Portion	Portion	Portion
	Pudding	,,	,,	,,
Feeds at night (when awake)	1. Whole milk (fresh or dried) . oz.	5	5	5
	2. 1 egg beaten up in milk . . oz.	5	5	5
Between feeds	Strained orange or tomato juice . oz.	1	1	1
	Approximate caloric value . . .	2,545	3,118	3,624

immediate feeding, supplemented when necessary by transfusion, are much superior to starvation or operation." The advocates of early feeding to the exclusion of the mouth starvation method must, however, remember that in Witts' original series of cases he found that not all the patients could take even his fluid diet from the start. The diet is as shown on p. 181.

The milk is not citrated, but may be flavoured with Horlick's malted milk or Ovaltine. Vitamin C is given as fruit juice or as ascorbic acid tablet 50 mg. t.i.d. Sips of water or of dextrose solution or half strength normal saline are allowed between feeds, up to 5 oz. an hour. The total fluid intake in the twenty-four hours should be about 5 pints. Neutralising substances or alkalis are only given if there is abdominal pain, which is unusual. No purgatives are given, and if the bowels are not opened an enema is given on the seventh day. Transfusion is given according to the criteria laid down above (see p. 179). Woldman (22) treated 21 cases of hæmatemesis, admitted to St. Luke's Hospital, Cleveland, Ohio, by the continuous intragastric aluminium hydroxide drip method (see p. 188), and they all recovered. In addition, during the first twenty-four hours the patients were given by mouth 2 oz. of milk and cream every two hours, and after that the diet included cooked cereals, gelatine, custard, cream soup, and rice and tapioca puddings. Morphine was not used, but barbiturates and other sedatives were administered as required. Woldman considers the drip is a further advance on the principle of early feeding. This compares favourably with the 38 cases of hæmatemesis, admitted to the Hospital during the previous five years, who were not treated by the drip, for 11 of these cases died.

The Surgical Treatment of Hæmatemesis. Gordon-Taylor (23) urges that the surgeon should not be excluded from consultation until the last desperate stage of ulcer hæmorrhage, and supports the view expressed by Finsterer (24) that the first forty-eight hours is the optimum period for operation. The practical difficulty is to decide in any individual case whether death will occur if medical treatment is adopted, and whether life could be saved by an early operation. Further, it is often impossible to decide at this stage whether the patient has an acute or chronic ulcer or chronic gastrititis. The chief indications for operation are (*a*) The erosion of a large vessel. (*b*) Concomitant hæmorrhage

and perforation of the stomach. (*c*) Hæmorrhage which cannot be controlled by blood transfusion. (*d*) A primary hæmorrhage may require surgical treatment in elderly people whose vessels are thickened and blood pressure raised. An operation is rarely, if ever, advisable in primary hæmorrhage under other circumstances, and acute ulceration of the stomach probably never requires surgical treatment.

The position with recurrent hæmorrhages is somewhat different. Previous X-ray investigation will probably have been carried out. With a large penetrating ulcer, a fibrotic ulcer, with pyloric or duodenal stenosis or with mid-gastric narrowing, an operation may be required to prevent further bleeding. It should not be performed until the hæmoglobin has been raised to 60% by blood transfusion. In no case should an operation be advised unless very skilled surgery is available with adequate facilities for blood transfusion, for the mortality rate for operation for active hæmorrhage from chronic ulcer is at the best about 10%. In all cases in which the patient is under forty-five years of age an operation is best avoided.

The Medical Treatment of Ulcers not Complicated by Hæmorrhage. The modified Sippy treatment is now usually employed.

The Sippy Treatment (modified) or The Alkali Treatment. Sippy (25), of Chicago, introduced this treatment in 1915. Its object is to protect the ulcer from hydrochloric acid. This is accomplished by giving large doses of alkalis after, and of belladonna before the feeds, and by using as the basis of the diet milk and cream.

Modifications of the Sippy treatment have been found to yield good results and are more easily carried out than the original method. The feeds are given at two or two and a half hourly intervals, an alkaline powder or neutralising substance is taken one hour after, and olive oil or belladonna before the feeds. There is some difference of opinion as to the best alkalis or neutralising substances for the purpose. Sippy (25) recommended sodium bicarbonate, heavy magnesia and bismuth carbonate. All carbonates have the disadvantage of liberating CO_2 and causing gaseous distention, which proves distressing in some cases. Sodium bicarbonate and magnesium oxide provoke a secondary secretion of gastric juice. Bismuth carbonate has no neutralising power

and theoretically is of little value, as it is improbable that it forms a protective coat to the ulcer. It is, however, out of fashion now owing to its expense, to the slaty colour it gives to the fæces which interferes with the detection of blood, and because it is said neither to relieve pain nor to neutralise acidity. Clinically, however, bismuth undoubtedly exerts a sedative effect. Magnesium oxide and magnesium hydroxide are both powerful neutralising agents. Sodium and potassium citrate, tribasic calcium phosphate and tribasic magnesium phosphate neutralise hydrochloric acid without producing an alkaline solution (26), (27). Crohn (28) in 1929 introduced colloidal aluminium hydroxide as an antacid. Six daily doses of 60 to 120 m. of a preparation such as Aludrox (5·8% aluminium hydroxide) in water may be given between feeds. An alternative preparation is Creamalin containing 5·5% aluminium hydroxide, the dose being 120 m. in water between feeds. Fauley et al. (29) have shown that aluminium hydroxide gel in relatively large doses interferes with the absorption of phosphates in man and dog, and that it may produce a phosphorus deficiency in the presence of a relative deficiency of pancreatic juice, of diarrhœa or of a low phosphorus diet. This is, however, not likely to occur with a patient on an ordinary ulcer diet. They also showed that aluminium phosphate gel will prevent the formation of a peptic ulcer in Mann-Williamson dogs (in which a gastro-jejunostomy is performed and the pancreatic juice and bile are diverted into the last 20 to 25 cm. of the ileum). In such dogs a jejunal ulcer develops with death in about eleven weeks. Aluminium phosphate gel does not interfere with phosphate absorption. Aluminium hydroxide gel did not prevent ulcer formation in these dogs. The use of aluminium phosphate gel in a few cases of patients suffering from peptic ulcer indicated that it was as effective as any other neutralising agent, and it is probably preferable to aluminium hydroxide gel in cases associated with pancreatic deficiency or diarrhœa. Mutch (30) has obtained good results with a synthetic hydrated magnesium trisilicate in doses of gr. 5 to 21 (anhydrous weight) between each feed. It possesses antacid and general adsorbent properties. Cohn et al. (31) record the case of a granuloma of the stomach following the ingestion of a medication containing hydrated aluminium silicate which the patient had been taking for a long time for a duodenal ulcer.

There is some risk of producing alkalosis if large doses of magnesia or sodium bicarbonate are given, but this risk is diminished if citrates, phosphates or colloidal aluminium hydroxide are employed. The powder which we have found useful is magnesium hydroxide, prepared chalk and bismuth carbonate in equal parts. The amount of magnesium, calcium and bismuth salts can be varied according to whether a laxative or astringent effect is necessary to secure an efficient action of the bowels. Alternatively we prescribe Aludrox.

Milk is the best neutralising food substance, and the addition of sodium citrate, gr. 3 to the ounce, not only prevents curds forming, but also increases the neutralising effect. Fatty substances tend to diminish gastric secretion, carbohydrates have little neutralising power.

During the stage of healing of the ulcer the patient must be kept in bed and feeds given at not more than two to two and a half hourly intervals during the day; by night a feed should only be given if the patient wakes. The time for an ulcer to heal, as judged by X-ray appearances and negative tests for occult blood in the fæces, varies according to its size from four to eight weeks. It is improbable that a deeply excavated and adherent ulcer could heal so quickly. Although some authorities maintain that it is irrational to increase the diet until the ulcer is healed, in our experience this can be done with advantage by carefully graduated changes.

The treatment we adopt is as follows :—

Healing Stage (Diet 1). *Weeks* 1 *and* 2. Feeds of 5 oz. are given at 7 a.m., 9 a.m., 11 a.m., 1 p.m., 3 p.m., 5 p.m., 7 p.m., 9 p.m., 11 p.m., and once during the night, if awake. The feeds consist of milk (warm and containing 3 gr. sodium citrate to the ounce), Horlick's malted milk or Benger's food. At least five of the feeds should be of milk which may be coloured with tea. Strained orange or tomato juice, 1 oz., should be given daily throughout the treatment to supply vitamin C.

Week 3. Add one raw egg to two of the milk feeds. A little thin bread and butter is given with one feed, and cream 1 oz. three times a day. In addition, the patient may be given once a day a little of one of the following : sweet jelly, milk jelly, custard or junket. The feeds are still given two-hourly.

Week 4. The feeds are given every two and a half hours, from 7 a.m. to 10 p.m. They consist of 5 oz. of citrated milk every other feed with one egg in two of the feeds, the thin bread and butter and the cream, as in week 3. The alternate feeds are composed of a similar quantity of potato soup, arrowroot, cornflour, or milk pudding (sago or tapioca), and once a day a little sweet jelly, milk jelly, custard or junket.

Week 5. The feeds are given every two and a half hours. The milk may now be reduced to three times a day, at 7 a.m., 5 p.m. and 10 p.m. Additions to the diet are pounded fish 2 oz., lightly boiled egg, crisp toast or rusk (well chewed) with butter.

Weeks 6 *to* 8. Feeds still every two and a half hours. Add 2 to 4 oz. pounded fish or minced meat daily.

Take immediately before three feeds, $\frac{1}{2}$ oz. of olive oil, and immediately before three other feeds, tinct. bellad. m. 5–10, aq. chlorof. ad fl. oz. $\frac{1}{2}$, or atropine sulphate gr. $\frac{1}{200}$ in m. 60 of water. Take one teaspoonful of the alkaline powder or of Aludrox one hour after five feeds during the day and two teaspoonfuls the last thing at night, in a little water, during the first two weeks of the treatment.

During the third and fourth weeks take a teaspoonful of the powder or of Aludrox three times a day after feeds and two teaspoonfuls at night. In the fifth to eighth weeks take a teaspoonful of the powder or of Aludrox twice a day after feeds and two teaspoonfuls at night. The mouth should be cleaned after each feed with bicarbonate of soda solution, gr. 60 to 5 oz. of water. This is important, as parotid suppuration may occur if it is neglected. No smoking is allowed.

Convalescent Stage (Diet 2). During this period of about a month the patient is allowed up for gradually increasing periods, and additions are made to the dietary.

7.30 *a.m., Breakfast.* One egg (lightly boiled), thin bread and butter or crisp toast and butter (to be well chewed), honey or apple jelly, sugar and milky tea.

10 *a.m.* Milk 5 oz. (containing 15 gr. sodium citrate) or Horlick's malted milk or Benger's food.

12.30 *p.m., Lunch.* Milk soup (potato or artichoke), fresh white fish (boiled or steamed), or rabbit, chicken or tender mutton,

mashed potato. Custard or milk pudding or stewed apples (with no pips, skin or core), and the juice of an orange.

3 *p.m.* Thin bread and butter or biscuits and butter and milky tea.

5.30 *p.m.* Milk 5 oz. (containing 15 gr. sodium citrate) or Horlick's malted milk.

8 *p.m.*, *Dinner.* As for lunch.

10.30 *p.m.* Milk 5 oz. (containing 15 gr. sodium citrate) or Benger's food.

The alkaline powder or Aludrox should be taken, one teaspoonful after breakfast and two teaspoonfuls last thing at night, and the olive oil or belladonna before breakfast, lunch and dinner. The meals should be small and eaten slowly. No smoking is allowed.

After Treatment (Diet 3). 8 *a.m.*, *Breakfast.* A selection from porridge, made from very fine oatmeal; egg, boiled, poached or scrambled; toast, crisply made and well chewed; breakfast biscuits; honey; apple jelly; butter; sugar; cream; and milky tea or coffee.

11 *a.m.* A tumbler of milk, Ovaltine, Horlick's malted milk, egg and milk, or Benger's food and a biscuit.

1 *p.m.*, *Lunch.* A selection from soup, made from milk and vegetables, such as potato or artichoke. Fresh white fish, boiled or steamed with white sauce, or fish soufflé. Meat, such as minced fresh beef, mutton or veal, grilled lamb cutlet, boiled or roast chicken. Vegetables, such as mashed potato, steamed or boiled green vegetables, passed through a sieve and served with butter. Sweets, such as custard, boiled or baked, junket, milk pudding very well cooked, cornflour, stewed apples with no pips, core or skin, chocolate soufflé. Crisp toast, the juice of one or more oranges, cheese soufflé.

4 *p.m.* A cup of milky tea, thin bread and butter.

7 *p.m.*, *Dinner.* As for lunch.

10 *p.m.* A feed as at 11 a.m.

The breakfast, lunch and dinner should be small meals, well chewed and eaten slowly. The patient should rest for half an hour after meals. No condiments should be eaten, and nothing taken which cannot be reduced to a soft pulp in the mouth. If it is impossible to obtain the liquid feeds between all the meals,

a biscuit or some chocolate should be eaten. A teaspoonful of the alkaline powder or of Aludrox should be taken every night on retiring to bed. No smoking is allowed.

The following articles of food are forbidden : Smoked salmon, tough meat, high game, sausages, curry, made-up dishes, fried foods, cheese (except cream cheese), meat extracts and meat soups, pickles, salads, uncooked vegetables, new or wholemeal bread, buns, unripe or raw fruit, nuts, raisins, jam with pips, marmalade with peel, strong tea or coffee, aerated drinks and alcohol.

The Danger of Alkalosis. Large doses of alkalis, especially of sodium bicarbonate, magnesium oxide and carbonate, may produce toxic symptoms (Hardt and Rivers (32)). Alkalosis is liable to occur in cases of gastric stasis especially associated with duodenal ulcer or renal insufficiency. It would appear that this observation is difficult to reconcile with the rationale of the alkali treatment of nephritis, for renal insufficiency usually tends to produce acidosis. The symptoms are headache, vertigo, loss of appetite, distaste for milk, malaise, nausea, vomiting, drowsiness or coma. A determination of the alkali reserve of the blood is of value in confirming the diagnosis. In the early stages the symptoms are usually relieved as soon as the alkali is stopped. Hardy (33) reports a severe case, in which the headache began the day after the treatment commenced, and on the fourth day the patient was desperately ill. The urine contained albumin and granular casts, the blood pressure and blood urea were raised. In such a case all alkalis should be stopped, dextrose given by mouth or in rectal salines, ammon. chlor. 0·5 g. (stearettes) should be administered by mouth every six hours, or by rectum in doses of 4 g. every six hours, and an intravenous injection made of collosol calcium (1 ml.), repeated next day if necessary.

Continuous Intragastric Drip Treatment. Winkelstein (34) in 1932 determined the gastric acidity during the night in cases of duodenal ulcer, gastric ulcer and normal controls. A Rehfuss tube was inserted in the stomach at 7 p.m. and specimens of gastric juice removed every two hours during the night. He found a high continuous acid secretion in cases of duodenal ulcer, a moderate acid secretion in gastric ulcer, but in the controls the

curve was very low, and in 4 out of the 6 cases no free hydrochloric acid was secreted during the night. He introduced a continuous Murphy drip through a Rehfuss tube for the treatment of peptic ulcer, using alkalinised milk. Woldman and Rowland (35) in 1936 published their results in cases of peptic ulcer treated with a continuous intragastric drip of 200 ml. of 7 per cent. colloidal aluminium hydroxide mixed with 600 ml. of water. The drip flowed at about 5 to 6 drops a minute, using up the 800 ml. in about twenty-four hours. By this means a constant achlorhydria resulted day and night. A small Levin tube, size 12, was passed through the nose into the stomach. These authors (36) reported further that gastric and duodenal ulcers which were quite apparent radiologically disappeared after about seven to fourteen days treatment; further, only 3 or 4 small bland feeds a day were required such as milk, cream, junket, custard, jelly, cereals, etc. Rutherford and Emery (37) in 1939 and 1940 used Creamalin (5·5 per cent. aluminium hydroxide), 1 part, to 3 parts of water, as a drip, running at 15 drops a minute. They treated 28 severe cases of peptic ulcer, which had previously failed to respond to medical or surgica treatment or to both. They gave the drip usually for a week and subsequently the aluminium hydroxide was administered by mouth for several months. In a follow-up fifteen months later, of 14 very severe cases of their original 28, they reported that 8 were well, 2 doubtfully improved and 4 were unsatisfactory. Winkelstein originally used a mixture of milk and sodium bicarbonate, but this is contra-indicated in cases of pyloric obstruction or renal disease owing to the risk of alkalosis, and if the patient is intolerant of milk. It was originally given continuously day and night for two or three weeks. Winkelstein et al. (38) writing in 1942 summarise their ten years' experience with the intragastric drip. They now use aluminium phosphate gel diluted with 4 volumes of water. It is less viscid and less constipating than aluminium hydroxide; it is run in at 15 to 20 drops a minute, using a soft latex tube, passed into the stomach through the mouth. A silkworm gut stilet, which extends to about 2 inches from the end of the tube, acts as a guide while passing the tube. The length of the tube to be passed can be gauged by measuring from the xiphoid cartilage up the mid-line to the lips. The tube is fixed by strapping to the cheek, and the

rubber tube connecting the drip apparatus (see Fig. 3) must be long enough to allow the patient to turn comfortably in bed. The patient soon learns to pass the tube himself, and it is used from one hour after the last meal to one hour before the next meal and during the night. Three liberal bland meals are given daily with atropine sulphate and sedatives as required. After three or four weeks it is only used at night and this continued for another six weeks. When the symptoms are mild the patient can be up and have the usual ulcer treatment by day and the drip by night, and thus avoid interference with work. The authors have treated successfully by this method several severe cases which had proved refractory to the usual Sippy treatment. Many of these were elderly men with histories of ten to thirty-five years of chronic penetrating ulcers, who had exacerbations of severe nocturnal pain, usually radiating to the back. The intragastric drip method of treatment is now extensively adopted in this country. A. Morton Gill has had a good experience of the method and reports as follows (personal communication). He uses a mixture of milk 1 pint, dextrose 2 oz. and sod. citrate gr. 10. A Ryle's tube is passed through the nose into the stomach and joined by a glass connection to rubber tubing attached to the drip. The fluid flows at the rate of 40 drops a minute, day and night, *i.e.*, 6 pints in twenty-four hours. If the tube blocks it is flushed through with a syringeful of milk. A specimen is removed every four hours for the first twenty-four hours, and titrated for free HCl; if it is present $\frac{1}{2}$ fl. oz. of Aludrox is added to each pint of milk. If the patient is hungry the amount of dextrose is increased. The Ryle's tube is changed every other day, cleaned and boiled,

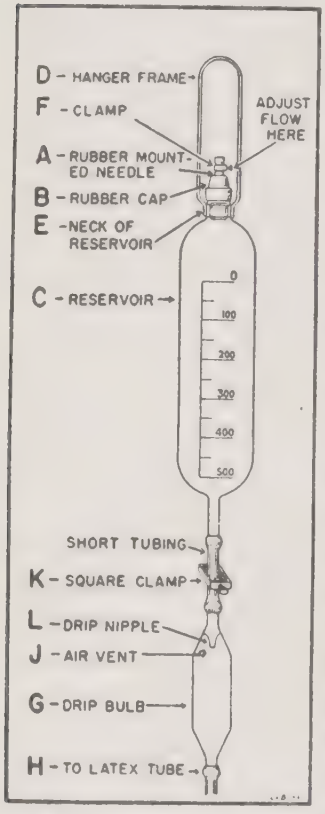

FIG. 3. Apparatus for intragastric drip. (Winkelstein, Cornell and Hollander, *Journ. Amer. Med. Assocn.*).

and the rubber tubing and glass connections are washed and boiled. Treating 150 consecutive cases of peptic ulcer, 6 were given the drip feeding because the ulcer did not heal after eight weeks strict treatment in bed on the conventional Sippy lines. Of these 6 cases, 2 with gastric ulcer and 2 with duodenal ulcer, healed after three weeks' drip feeding, one was unable to tolerate the tube, and one remained unhealed despite the drip treatment. In another series of 9 cases which had failed to heal with the usual medical treatment, 7 healed by the drip method in periods of three to seven weeks.

Whatever form of treatment is adopted, it is important that, as soon as the patient is in a fit state, a search should be made for any possible focus of infection, which should be eradicated when found. This is a proceeding similar to that which has to be adopted in the treatment of diabetes mellitus. The teeth should be X-rayed, the nose and sinuses examined, and pyorrhœa should be treated. A search for signs of appendicitis or infection of the gall-bladder or uro-genital tract should also be made.

The Medical Treatment of Chronic Ulcers. This is in all essentials similar to that detailed above for acute ulceration of the stomach or duodenum; in fact, apart from the history of the duration of the case, it is difficult to distinguish between the two conditions.

It will be noticed that throughout this section gastric and duodenal ulcers have been considered together, as if their treatment were the same. Duodenal ulcers are of much more frequent occurrence, and are more easy to diagnose.

The Surgical Treatment of Ulcers not Complicated by Hæmorrhage. While, on the one hand, it is clear that in certain circumstances medical measures are of no avail in the treatment of gastric and duodenal ulcers, yet patients are frequently seen who have had operations for these conditions and who have received no permanent benefit. Indeed, their last state is not infrequently worse than their first. It is, therefore, profitable to endeavour to decide what are the indications for surgical treatment. In this way patients should derive the maximum benefit possible, and not be subjected to operations which either give no relief or are followed by only a temporary amelioration of the symptoms.

The following are the chief indications for operation :—

Surgical treatment is imperative in cases of perforation or of perigastric abscess, although some cases of perforation have been successfully treated by continuous gastric suction.

Ogilvie (39) makes the following further recommendations :—

Gastric Ulcer. Operation is required for the following reasons : (*a*) Organic stenosis causing delay in emptying. (*b*) If cancerous transformation of the ulcer is suspected. (*c*) If medical treatment fails, as will occur if a chronic ulcer becomes fixed to neighbouring structures. The acid secretion in gastric ulcer is not usually raised, and gastrectomy is generally the most satisfactory operation.

Duodenal Ulcer. Operation is required for organic stenosis. A posterior gastro-enterostomy or a gastro-duodenostomy should be performed. As the gastric acidity is usually low, owing to the presence of chronic gastritis, recurrent anastomotic or jejunal ulceration is unlikely to ensue. Recurrent ulceration of this type is probably due to the operation of gastro-enterostomy being performed in the presence of high gastric acidity. Further, the average time for the appearance of the recurrent ulcer is several years. Gastro-jejunostomy alone is, therefore, absolutely contra-indicated when the gastric acidity is high and the rate of stomach emptying is rapid, with the following exception. In a patient over the age of fifty, if symptoms of duodenal ulcer persist, usually because the patient will not adhere strictly to his medical treatment, a short-circuiting operation may be performed, even if there is no stenosis. The gastric acidity tends to fall progressively after the age of fifty, and so the chance of a recurrent ulcer is slight. In younger subjects, with higher acidity, gastrectomy should be performed. Operation is always contra-indicated for acute duodenal ulcer, and for chronic duodenal ulcer in young patients, when there is no organic stenosis.

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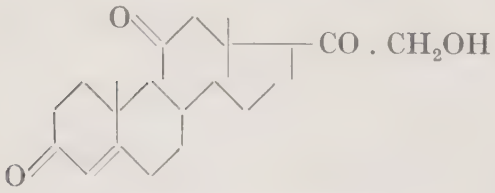
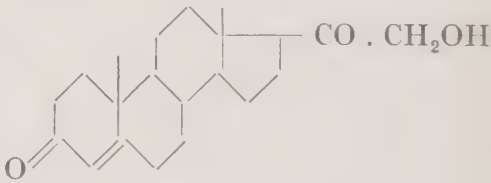
CHAPTER VII

THE TREATMENT OF ADDISON'S DISEASE

The Cortical Hormone. In 1931 Swingle and Pfiffner (1) prepared an extract of the cortex of the suprarenals which was capable of restoring moribund suprarenalectomised animals and of maintaining them over long periods in moderate health. The solution is standardised for its activity upon suprarenalectomised cats, the potency being judged by its power to keep the animal alive for a minimum period of thirty days. The standardisation is crude and it is impossible to speak of units with any degree of accuracy. Various preparations are available, such as Eucortone, Eschatin, and Cortin. Much work has been done on the constitution of the active extract of the suprarenals, and a full summary will be found in the paper by Reichstein (2). It has been shown that the nucleus of the substance is the same as that of the sex hormones and sterols, namely, cyclopentenophenanthrene. A large number of substances have been prepared from the suprarenal cortex, of which many are active. The formula of one of the most important, dehydro-corticosterone, is shown below.



Dehydro-corticosterone



Desoxy-corticosterone

A Synthetic Cortical Hormone. Steiger and Reichstein (3) have succeeded in preparing a synthetic analogue desoxy-corticosterone, and it has also been isolated from the suprarenal cortex. The formula is shown above. Desoxy-corticosterone acetate is dissolved in sesame oil, 5 or 10 mg. in 1 ml., and is available for therapeutic use as Percorten, Syncortyl, and D.O.C.A. Clinical experience indicates that 5 mg. of desoxy-corticosterone are equivalent to 10 to 15 ml. of a cortical extract such as Eucortone.

The Treatment of a Subacute or Chronic Case. The principles of treatment consist in the use of a cortical extract or a synthetic preparation, the administration of sodium chloride and water, and the restriction of potassium in the diet.

The Use of a Cortical Extract or of a Synthetic Preparation. A cortical extract, such as Eucortone, can be given intravenously, intramuscularly or subcutaneously. Intravenous administration is usually only necessary in severe cases. For a case of moderate severity 10 to 20 ml. are required daily, and the maintenance dose in an average case is 10 ml. every other day. More is usually needed in hot weather and in times of mental stress. In 1938 Simpson (4) reported on the use of the synthetic preparation in two cases of Addison's disease. The dose of the synthetic preparation, Percorten, is 5 to 10 mg. daily for a case of moderate severity, and the subsequent maintenance dose may be as little as 5 mg. weekly. Subcutaneous implantation of four pellets of 50 mg. each has been employed in some patients to avoid the necessity of frequent injections. The duration of the effect of the implants is uncertain, it is probably about two to three months, but Dunlop (5) found the beneficial effect, after the implantation of 200 mg., lasted for six to eight months. The chief advantage of the synthetic preparation is that its cost is about one-sixth to one-third that of the equivalent amount of cortical extract. Himsworth (6) and Avery Jones (7) have, however, reported instances of relapses in patients suffering from Addison's disease under treatment with desoxy-corticosterone acetate. This may be due to the absence of some factor in the synthetic preparation which is present in the cortical extracts.

The Administration of Sodium Chloride and Water. It has been noted that patients suffering from Addison's disease often have a craving for salt, and also they are frequently dehydrated. There is an excessive loss of sodium chloride and of fluid from the body which must be replaced. In some cases the administration of sodium chloride alone, without the use of a cortical substance, will cause marked improvement. The usual amount required is 5 g. t.d.s. This can be given by mouth in capsules, in milk or in water flavoured with syrup of lemon. Rynearson (8) recommends an "Addison's Elixir," which consists of sodium chloride 10 g., sodium citrate 5 g., water 1,000 ml., made palatable with a fruit-

flavouring extract. One to one-and-a-half litres are taken daily. We have found that most patients welcome the salt, they do not find it unpleasant to take and they feel much better when they are having it. Their weight increases and their blood pressure rises. Care must be taken that an over-dosage is not given over long periods, for Thorn and Firor (9) have shown that the continued administration of desoxy-corticosterone acetate with added sodium chloride may result in œdema, high blood pressure and even congestive heart failure.

The Restriction of Potassium Intake. Some authors such as Wilder, Kendall, Snell et al. (10) and de Wesselow and Thomson (11) have emphasised the importance of a low potassium intake in Addison's disease. The diet should contain less than 1·5 g. potassium daily, whereas a normal diet contains about 4 g. daily. A specimen diet as given by Wilkinson (12) is as follows: **Breakfast**: Egg, 1; butter, ½ oz.; bread (white), 2 oz.; cream, ½ oz.; marmalade; and tea. **Lunch**: Beef (stewed), 3 oz.; potato, 3 oz.; root vegetable, 4 oz.; pears or apples (tinned), 3 oz.; cream, 1 oz. **Tea**: Bread (white), 1 oz.; butter, ¼ oz.; honey, 1 oz.; cream, ½ oz.; biscuits (sweet); tea. **Supper**: Bread (white), 2 oz.; cheese, 1 oz.; butter, ½ oz.; cream, ½ oz.; tea or coffee.

This diet contains about 0·8 g. potassium. It is probably deficient in vitamins B_1 and B_2, calcium, phosphates, iron, etc. The meat and vegetables must be cut into small pieces and boiled in at least eight times their volume of water or in several changes of water to extract the mineral salts. Such a diet is difficult to prepare in an ordinary household and it does not appear to be a necessity in every case of Addison's disease, although in some it is definitely helpful. It usually suffices to avoid eating substances rich in potassium, especially peas, beans, lentils, soups, broths, gravies, dried fruits and chocolate, and to take only small quantities of meat and milk. Bread, cereals and sugar should be highly refined.

Treatment Before an Operation. Surgical operations, such as removal of a tuberculous kidney, can now be performed with comparative safety, whereas formerly they would have proved fatal. The patient, who has been stabilised with salt, a cortical preparation and a diet containing only a moderate amount of

potassium, is given on the morning of the operation an intravenous injection of 2 litres of a solution containing dextrose 10%, sodium chloride 1%, sodium citrate 0·5% and Eucortone 20 ml. This injection is repeated after the operation, once or twice, as required.

Treatment During a Crisis. The chief symptoms of a crisis occur in this order: progressive weakness, hiccough, vomiting, abdominal pain, diarrhœa and collapse. The patient appears dehydrated and cold and the blood pressure is low. Changes occur in the blood chemistry. Thus the urea may rise to 120 and the potassium to 26 mg. per 100 ml., whereas the chloride may fall to 440, the sodium to 260 and the sugar to 50 mg. per 100 ml. The patient should be put to bed and warmed with an electric cradle. A specimen of blood should be removed from a vein for the above analyses to be made. The blood pressure should be taken. A continuous drip intravenous injection is now started. The solution used consists of dextrose 10%, sodium chloride 1%, sodium citrate 0·5% and 15 ml. of Eucortone per 1,000 ml. water. Three to four litres are run in during the first twenty-four hours. Additional Eucortone up to 150 ml. in twenty-four hours may be required. After twenty-four hours the patient is usually very much better and is encouraged to drink fluids freely. He is then able to begin the treatment as detailed above for cases of moderate severity.

The Cutler Test in the Diagnosis of Addison's Disease. In some cases there is difficulty in the diagnosis of adreno-cortical insufficiency, especially when it is present in only minor degrees. The sodium chloride deprivation test of Cutler, Power and Wilder (12) is then helpful, but it must always be carried out with care, as the sudden deprivation of salt combined with the administration of potassium may provoke a crisis, which, unless treated promptly, may in turn prove fatal. It should, therefore, never be carried out on a case which, on clinical evidence, is clearly one of Addison's disease. In this test the patient is given a low sodium diet and the urinary sodium or chloride is estimated during the last four hours of a fifty-two-hour period. For practical purposes the chloride only is estimated. In Addison's disease, despite the low blood concentration of sodium chloride, there is a higher urinary concentration of sodium and chloride, on the average about six times higher than normal. Under the conditions

of the test a urinary chloride concentration in excess of 225 mg. per 100 ml. is indicative of abnormality of adreno-cortical function, whereas a concentration of less than 125 mg. per 100 ml. can probably be accepted as evidence of normality. During the period of dieting extra potassium is administered as it increases the sodium and chloride output.

The details of the test are as follows : no cortical extract nor extra sodium chloride must be given during the preceding twenty-four hours. The test begins at 8 a.m. on the first day and ends at noon on the third day. The urine for analysis is collected between 8 a.m. and noon on the third day. During the fifty-two hours of the test the following daily diet is given :—

Breakfast : Orange, 100 g. ; bread (salt free), 50 g. ; butter (salt free), 10 g. ; cream (20%), 25 g. ; coffee (medium), 300 g. ; egg, 1 ; jelly, 20 g. **Lunch** : Tomatoes (canned), 90 g. ; lettuce, 10 g. ; peaches, 100 g. ; potato (baked), 100 g. ; bread (salt free), 30 g. ; jello, 150 g. ; butter (salt free), 10 g. ; cream (20%), 25 g. ; coffee, 200 g. ; beef, lean (weighed before cooking), 75 g. ; jelly, 20 g. **Supper** : Lettuce, 10 g. ; grapefruit, 100 g. ; banana, 100 g. ; peas (canned), 100 g. ; potato (baked), 100 g. ; bread (salt free), 30 g. ; butter (salt free), 10 g. ; cream (20%), 75 g. ; milk, 200 g. ; beef, lean (weighed before cooking), 50 g. ; jelly, 20 g.

This diet contains potassium 4·062 g., sodium 0·592 g. and chloride 0·949 g.

Fluid Intake. On the first day this is unrestricted and encouraged. On the second day 18 ml. per lb. body weight, and on the third day between 8 a.m. and 11 a.m. 9 ml. per lb. body weight are given. Extra potassium in the form of potassium citrate (42 mg. per lb. body weight) is given at 4 p.m. on the first day and at 10 a.m. on the second day. At the conclusion of the test the patient should receive an intravenous injection of dextrose 50 g., sodium chloride 10 g., sodium citrate 5 g. and Eucortone 20 ml. in 1,000 ml. of water. Should a crisis be provoked by the test, give the intravenous injection at once.

We have found this test useful in the diagnosis of Addison's disease, especially in cases of pigmentation accompanied by asthenia, and in cases of pulmonary tuberculosis in which persistent vomiting occurs and it is uncertain whether or not the suprarenals have become the seat of tuberculous changes.

Results of Treatment. The use of these combined methods of treatment has materially improved the prognosis in Addison's disease. Patients have been restored to health sufficiently to carry on with their work for several years. Clinically, in addition to the return of strength, the weight and blood pressure usually rise and the pigmentation diminishes, although it is not likely to disappear completely. Operations can now be carried out with comparative safety. The prognosis is better when the adrenal lesion is a simple atrophy than when it is tuberculous. The prognosis is bad when active pulmonary tuberculosis is present, and in all cases in which the adrenal lesion is tuberculous, tuberculous deposits are very likely to be present in other parts of the body which may prove fatal. Intercurrent infections and very hot weather are further sources of danger.

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CHAPTER VIII

THE CARDIOVASCULAR SYSTEM

Cardiac Irregularities

Potain (1), in 1867, obtained tracings from the jugular vein in man. He used as a receiver of its pulsations a glass funnel which was connected by a rubber tube with a Marey sphygmograph, and recorded at the same time tracings from the apex beat, carotid and radial arteries. In 1892 Sir James Mackenzie (2), using a small metal cup as a receiver, devised the instrument originally called a phlebograph, by means of which simultaneous tracings of the pulsations of the jugular vein and radial artery were obtained. The records were taken upon smoked paper, but by the use of inked pens the polygraph was evolved.

Our knowledge of the essential nature of the different forms of cardiac irregularity is mainly due to Sir James Mackenzie's pioneer work with this instrument, by means of which he obtained records representing the activities both of the auricle and of the ventricle. Further light has been thrown upon this subject and upon myocardial disorders by the use of the electrocardiograph.

The chief varieties of cardiac irregularity are as follows: (1) Sinus arrhythmia; (2) premature systoles; (3) heart-block; (4) auricular flutter; (5) auricular fibrillation; (6) paroxysmal tachycardia; and (7) pulsus alternans.

The Electrocardiograph

The electrical currents produced by the contraction of the human heart were first recorded by Waller (3) in 1887 by means of the capillary electrometer. With this instrument the movements of a fine column of mercury are photographed on a moving plate.

The string galvanometer was later introduced by Einthoven (4) in 1903, and modifications of it are now known as the electrocardiograph.

Immersion electrodes have been largely replaced by small

electrodes of German silver plate, about 3 square inches in size. A cotton-wool pad wrung out in warm normal saline may be placed between the electrode and the skin, the electrode being held in position by a rubber band. Cambridge electrode jelly may be used instead of the wool pad ; a small portion is squeezed

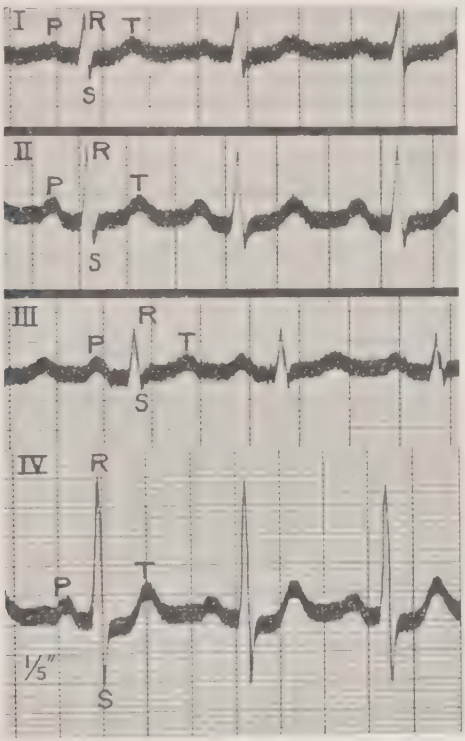

FIG. 4. Normal Electrocardiogram (Lead IV R, left arm electrode anteriorly, right arm electrode on right arm).

from the tube and rubbed gently into an area of skin, corresponding with the size of the electrode.

The leads usually adopted are as follows :—

Lead I (transverse). Right and left hands.

Lead II (axial). Right hand and left foot.

Lead III (left lateral). Left hand and left foot.

Lead IV (chest leads). See p. 213.

Curves representing the changes in potential caused by the heart's beat are photographed. A single beat from the normal heart shows the following waves (see Fig. 4) :—

P is due to auricular systole, and constitutes the auricular complex. **QRST** occur during ventricular systole, and constitute the ventricular complex. **Q** and **S** are often absent. **T–P** represents diastole.

Certain time relations are important :—

The **P–R** interval is normally 0·14 second. Prolongation of this indicates delay in conduction in the A–V node or the upper part of the bundle of His.

The **QRS** interval is normally just under 0·10 second. Prolongation of this occurs in the various grades of heart-block and in ectopic ventricular beats.

The **R–T** interval is normally 0·32 second. Prolongation of this occurs especially in complete A–V block.

The **QRST** curves are produced by currents originating in the two ventricles, and represent the algebraic sum of the waves produced by the two ventricles contracting separately.

The characteristic appearance of the deflections in the first three leads should now be studied (see Fig. 4). The **R** wave is normally greatest in Lead II, and $\mathbf{R}_1$ and $\mathbf{R}_3$ are together approximately equal to $\mathbf{R}_2$. (The figures below the waves indicate the leads in which the waves occur.) The **P** and **T** waves are also usually larger in Lead II than in Leads I or III.

The Significance of certain Alterations in the Form of the Deflections. *The P Wave.* This may be inverted, indicating that the impulse originates at a new focus in the auricle (at times in the A–V node as in paroxysmal tachycardia and in nodal extrasystoles). The **P** wave may be absent, or replaced by a small series of oscillations of a fine or coarse variety, as is seen in auricular fibrillation. It may be abnormally large and notched, indicating auricular hypertrophy, as may occur in mitral stenosis. $\mathbf{P}_3$ may be greater than $\mathbf{P}_2$, as may be seen in auricular flutter.

The R Wave. A maximum **R** wave in Lead I indicates left axis deviation, which, when present to a high degree, is most often due to left ventricular preponderance. It may also be associated with an unduly transverse lie of the heart, associated with a high diaphragm. In right axis deviation, which also includes right ventricular preponderance, the **R** wave is greatest in Lead III.

Normally the left ventricle is 1·8 times heavier than the right.

The S Wave. A maximum S wave in Lead I indicates right axis deviation, whereas a maximum S wave in Lead III is a sign of left axis deviation.

The T Wave. It is held that the T_2 wave is of great importance as indicating the condition of the ventricular muscle. A negative T_2 wave is always pathological and a negative T_1 wave usually indicates a pathological condition. With full doses of digitalis the S–T segment is usually depressed and the T wave inverted in all leads. A negative T_3 wave is not necessarily pathological.

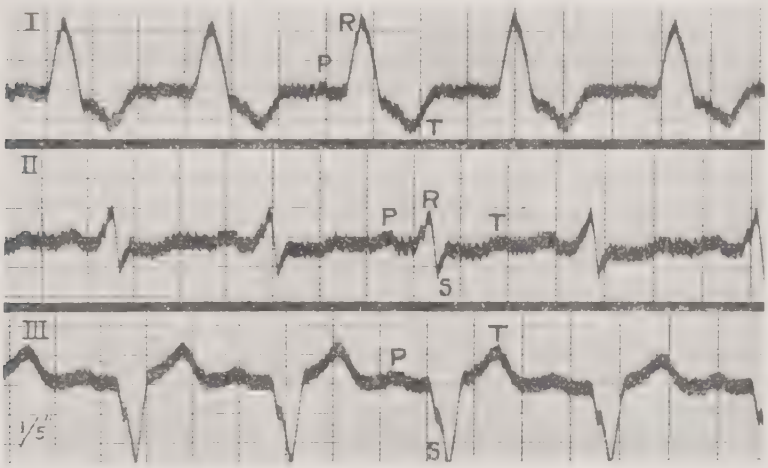

FIG. 5. Bundle-Branch Block (Type I).

Prolongation of the QRS Complex. In the condition known as bundle-branch block, the right or left branch of the bundle of His is impaired and fails to conduct. The **QRS** complex is prolonged or spread out, and lasts longer than the normal maximum of 0·10 second. The **R** wave may be notched. The **T** wave is in the opposite direction to the main initial deflection (**R** or **S**), and is usually of a greater amplitude than normal. The ventricular complex is therefore diphasic.

Two types of curves are met with in patients suffering from bundle-branch block, depending on which branch of the bundle is obstructed. The old classification was based on animal experiments, and according to the type of curve obtained right or left bundle-branch block was diagnosed. Later work on human subjects has thrown doubt on the applicability to man of these

conclusions drawn from animal experiments, and there is therefore uncertainty as to which type of curve corresponds with right or left bundle-branch block. The curves are referred to now simply as Type I and Type II.

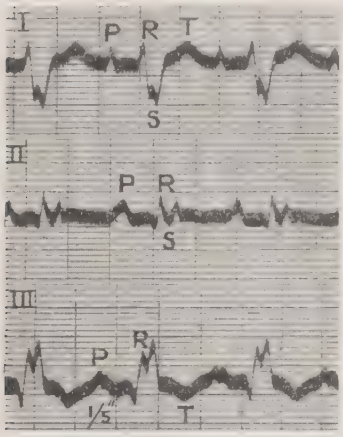

FIG. 6. Bundle-Branch Block (Type II).

Type I Bundle-Branch Block (old terminology, right). The main initial deflection (**R**) is positive in Lead I and negative (**S**) in Lead III (see Fig. 5). This is the common variety.

Type II Bundle-Branch Block (old terminology, left). The main initial deflection (**S**) is negative in Lead I and positive (**R**) in Lead III (see Fig. 6).

Type IIa. This was described by Wilson and his co-workers (5) in 1934, and in 1937 was called by Evans and Turnbull (6) "the newer electrocardiogram denoting right bundle-branch block." It is more common than the hitherto

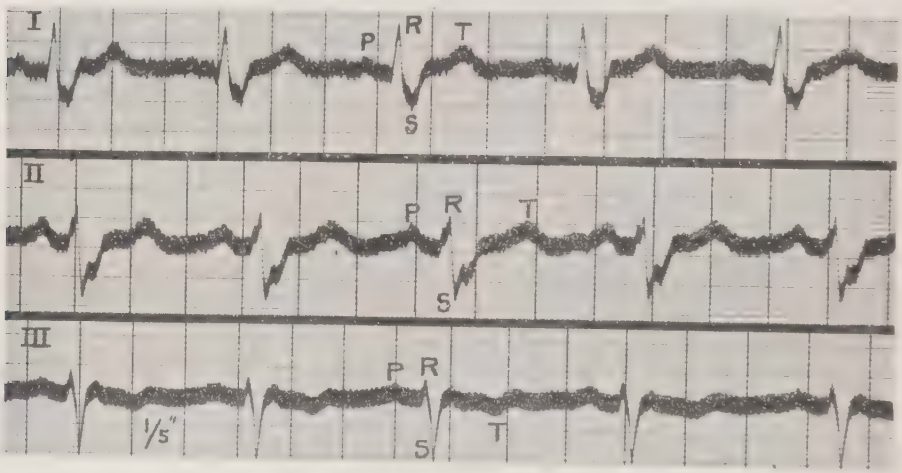

FIG. 7. Bundle-Branch Block (Type IIa).

standard Type II curve. The distinctive features are: Lead I. **R** and **S** almost equal in size. **R** narrow. **S** broad and notched. **T** upright. Lead II. **S** broad and deep. **T** usually upright. Lead III. **Q** or **S** usually deep. **T** inverted (see Fig. 7).

The **QRS** complex is also prolonged in *intraventricular block*, in

which the terminations of the bundles in the ventricular subendothelial tissue are destroyed. The **R** wave is also often notched, and the **T** wave is in the same direction as the initial main deflection.

In *congenital dextrocardia* all the waves in Lead I are inverted, whereas Lead II may represent the normal Lead III, and *vice versâ*.

Absence of the PRT Complex. This occurs in sino-auricular

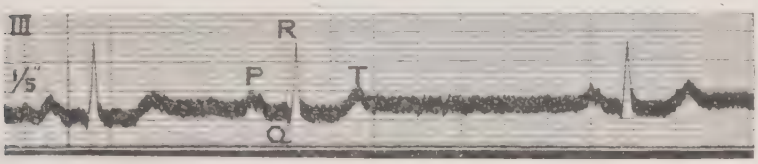

FIG. 8. Sino-Auricular Block.

block, in which the stimulus arising in the S–A node at times fails to cause a contraction of the auricle. The whole heart, therefore, misses a beat, but the succeeding contraction occurs at approximately the normal interval. This is not necessarily a serious condition (see Fig. 8).

In addition to the light which the electrocardiograph throws upon the condition of the myocardium and conducting tissue,

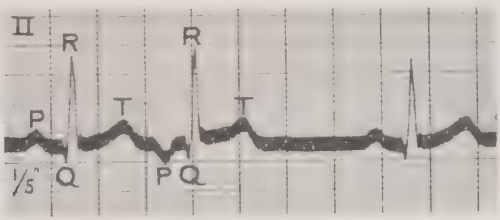

FIG. 9. Premature Auricular Systole.

the various types of cardiac irregularity can be accurately determined.

Sinus Arrhythmia. Variations in the length of the **T–P** intervals are seen, but the auricular and ventricular complexes are normal.

Premature Systoles. *Auricular.* The premature impulse may arise at the S–A node, near it, or some distance away. In the two former instances the **P** wave caused by the premature con-

traction is in the normal direction, in the latter it is inverted, indicating that the impulse spreads through the auricle along abnormal paths (see Fig. 9). The ventricular complex following the premature auricular stimulus is usually of normal type. The premature **P** wave may occur so early in diastole that it fuses with the previous **T** wave.

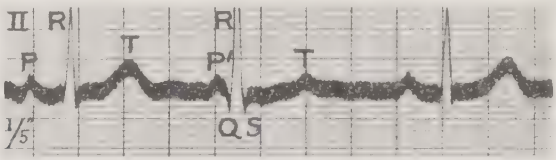

Fig. 10. Premature Nodal Systole.

Usually the diastole following the premature beat is not fully compensatory.

Nodal. The premature impulse arises in the junctional tissue uniting the auricle and ventricle, *i.e.*, in the A–V node or the A–V bundle before division. The ventricular contraction may occur just before, or abnormally shortly after, the auricular, but is seen to be supra-ventricular in origin because the **QRS** complex is of normal appearance (see Fig. 10).

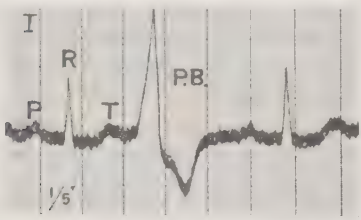

Fig. 11. Premature Ventricular Systole.

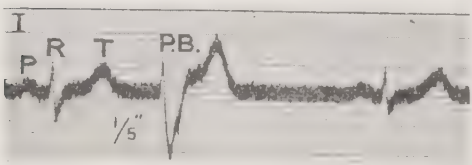

Fig. 12. Premature Ventricular Systole.

Ventricular. A premature stimulus arises in the ventricle below the division of the main A–V bundle. The premature ventricular complex resulting has the same duration as a normal ventricular complex, but its form is altered, the initial deflections of the premature beat being larger than normal. The auricular waves occur at regular intervals, and may be fused with the premature ventricular deflections, or appear just after them. The premature

ventricular beat is followed by a prolonged diastole and the succeeding pause is thus fully compensatory.

Two types occur (see Figs. 11 and 12) formerly called left and right ventricular premature systoles. As with the site of bundle-branch block, the localisation of ventricular systoles is now a matter of doubt, but of little clinical significance. According to the old terminology, based on experiments on dogs, the main ventricular deflection is upright in Lead I and inverted in Lead III with left ventricular premature systoles and with right bundle-branch block. With right ventricular premature systoles and with left bundle-branch block the deflections occur in the reverse direction. Barker, McLeod and Alexander (7) in 1930 introduced the new terminology which is the exact opposite of the old interpretation.

Heart-block. Various degrees of impairment of conductivity

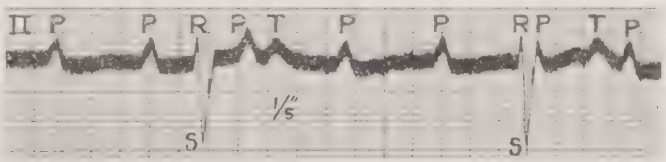

Fig. 13. Complete Heart-block.

of the heart muscle can be distinguished by the electrocardiograph.

Slight. Prolongation of the **P–R** interval to more than 0·2 second.

Occasional Dropped Beats. The **P–R** interval gradually becomes longer until, finally, the ventricular complex fails to follow its preceding auricular complex. The **P** waves are quite regular, but the ventricular complexes occur at different intervals, and the **P** and **T** waves may at times fuse.

Regular Dropped Beats. Every fourth, third or second ventricular contraction may be omitted, constituting a 4 : 3, 3 : 2, or 2 : 1 heart-block. Other varieties include 8 : 7, 7 : 6, 6 : 5, 5 : 4, 3 : 1, 4 : 1 and 5 : 1 heart-block. The first figure in each couple indicates the number of auricular, and the second the number of ventricular beats.

Complete Heart-block. The ventricle contracts at its own independent rate of about 30 beats a minute. The **P** waves occur regularly, and may be seen isolated on the electrocardiogram or

fused with **R** or **T** waves. The ventricular complex is of normal duration and configuration, and so the impulses giving rise to it are supraventricular, but as they do not originate in the auricle, they must be derived from the junctional tissue (see Fig. 13).

Bundle-branch Block and *Intra-ventricular Block* have already been described (p. 204) (see Figs. 5, 6 and 7).

Sino-auricular Block (*Tortoise Heart*). Some of the impulses

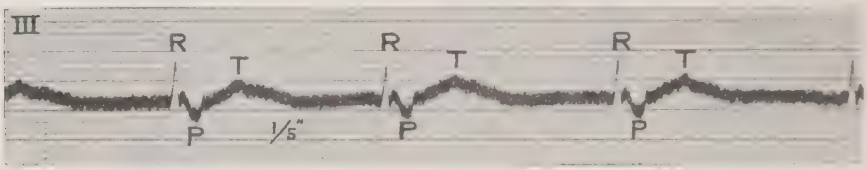

Fig. 14. Nodal Rhythm.

arising in the S–A node fail to reach the main mass of auricular muscle, and occasional intermittences of the whole heart may occur (see Fig. 8). If the rate is slow, as is usually the case, on exercise it may increase to about double.

When the heart is beating at such a slow rate the ventricle may interpolate a beat on its own (*ventricular escape*), the **P–R** interval being shortened, or the **P** and **R** waves may partially fuse.

Nodal Rhythm. The impulse may arise at the A–V node instead of at the S–A node, the auricles and ventricles contract simultaneously, and no **P** waves are seen or they occur shortly after the **R–S** complex and are usually inverted (see Fig. 14).

Auricular Flutter. An electrocardiogram shows the auricular

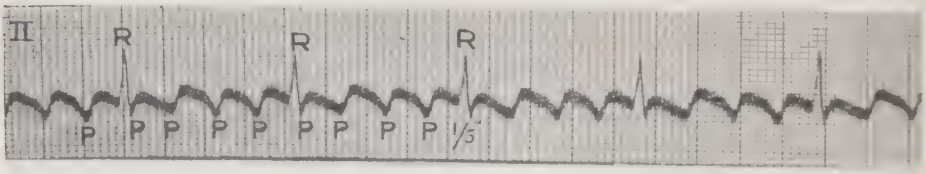

Fig. 15. Auricular Flutter.

contractions occurring at a regular rate, with ventricular complexes superimposed upon the curve at regular or irregular intervals. Heart-block of varying grades is almost invariably present.

The **P** waves occur at regular intervals and have a dome-shaped appearance. (See Fig. 15.) In some tracings the **T** wave is also apparent.

Pressure on the vagus in the neck may slow the rate of ventricular contraction, but the **P** waves continue to come through at regular and rapid intervals.

The auricular contraction is believed to pursue an abnormal circular path in the auricle. When the normal rhythm is established, as the result of the administration of digitalis or of quinidine, the gentle undulations of the **P** waves of flutter are replaced by the sharper peaked waves of normal rhythm.

Auricular Fibrillation. Here the **P** waves are absent, **QRS** deflections occur at irregular intervals, and are of varying height. The ventricular complex is of normal form. Oscillations of irregular size may be seen in diastole, caused by the fibrillating

FIG. 16. Auricular Fibrillation.

auricle ; these may be fine or coarse. They are usually best seen in Leads II and III. They also occur during systole, and may alter the shape of the **T** wave, although the **QRS** deflections are not affected. In rapidly beating hearts the oscillations are usually not well seen in diastole, but their effect upon the **T** wave is generally noticeable (see Fig. 16).

The **QRS** deflections may occasionally be seen at regular intervals, although the auricle is fibrillating and no **P** waves are present, but oscillations due to fibrillation may be seen in diastole. This occurs when the ventricle has adopted its independent rhythm owing to complete heart-block.

Ectopic beats may arise during fibrillation ; they are ventricular in origin. They may occur regularly after each **QRS** deflection, due to a supraventricular stimulus, constituting the coupled beats. This is usually due to excessive digitalis administration, and is a sign for immediate discontinuance of the drug.

Ventricular Fibrillation. Although compatible with life in man, if only of short duration (Gallavardin and Berard (8)), it is the probable cause of sudden death from heart failure, such as occurs under anæsthetics, etc.

Paroxysmal Tachycardia. *Simple Paroxysmal Tachycardia* (*Auricular*). A succession of ectopic auricular contractions occurs, arising at a new focus in the auricle. This is shown in the electrocardiogram by a rapid regular rhythm of about 150, in which the ventricular complexes are normal. The impulse has, therefore, a supraventricular origin. The **P** wave is often inverted in Leads II and III, and usually modified in Lead I. The auricular impulse is therefore ectopic in origin, and if not inverted in Leads II and III it is usually smaller than normal (see Fig. 17).

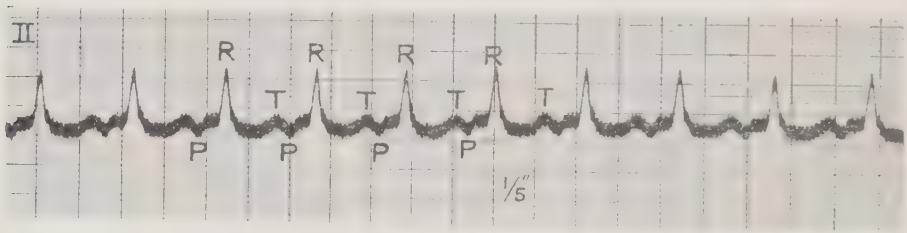

Fig. 17. Simple Paroxysmal Tachycardia, showing inversion of the "P" wave.

When the paroxysm ceases there is a pause, which is immediately followed by the restoration of the normal rhythm.

Auricular Flutter (see p. 208).

Auricular Fibrillation (see p. 209).

Nodal, arising in the A–V node. Here the auricle may contract first and the **P–R** interval be shortened, and **P** inverted, or the auricle and ventricle may contract simultaneously and the **P** and **R** be fused.

Ventricular. A rare condition characterised by a succession of ectopic ventricular systoles.

Pulsus Alternans. This condition, which is revealed by a tracing from the arterial pulse, is not always shown by the electrocardiograph. Thus, the **R** waves may be of equal height although the pulse shows marked alternation. At times the **R** waves may show alternation, but the large **R** waves may correspond with the small pulse waves, and *vice versâ*.

Occasionally the electrocardiogram shows alternation of the R waves, although the pulse tracing is regular.

Coronary Thrombosis. Besides disturbances in rhythm, coronary thrombosis often causes characteristic changes in the form of the ventricular complexes. These changes were first observed by Smith (9) after experimental coronary occlusion in dogs, and later by Herrick (10) and Pardee (11) in man. Rothschild and his co-workers (12) and Parkinson and Bedford (13) have demonstrated that serial records, at intervals after coronary thrombosis, show that the electrocardiographic changes usually occur in a definite sequence, corresponding with the pathological events in progress in the myocardium.

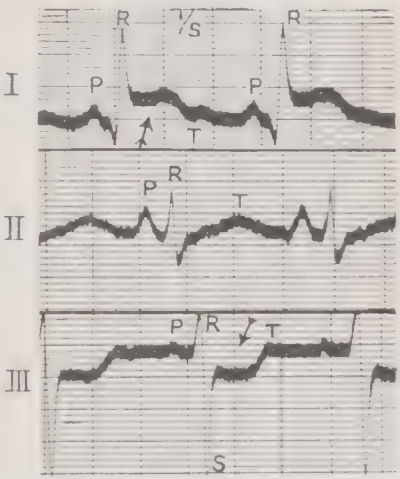

FIG. 18. Electrocardiogram taken four days after coronary thrombosis, showing plateau type of curve; the R–T period is elevated in Lead I and depressed in Lead III, as indicated by arrows. (Parkinson and Bedford.)

R–T Deviation. Within a few hours up to a week of the onset, the T waves take off from the RS waves above or below the zero level, so that RS is succeeded by a plateau-shaped elevation or depression (see Fig. 18). R–T deviation is most marked in Leads I and III, and is in opposite directions in these two leads; thus R–T elevation in Lead I is associated with R–T depression in Lead III and *vice versâ*.

T Wave Changes. Within about a week, the R–T segment returns to the iso-electric level and T waves develop, pointing away from the direction in which R–T was displaced in each lead. Thus R–T elevation is succeeded by an inverted T, and R–T depression by an upright T. Inversion of T occurs either in Lead I or Lead III, but not in both, and often to a lesser degree in Lead II. According to the direction of R–T displacement, and consequently to the direction of the T waves in Leads I and III, two types of curve are common.

1. *T1 Type.* T is deeply inverted in Lead I, often slightly

inverted or flattened in Lead II, and upright in Lead III (see Fig. 19).

2. *T3 Type.* T is deeply inverted in Lead III, often slightly inverted or flattened in Lead II, and upright in Lead I (see Fig. 20).

Changes in the direction of normal usually follow in the course of the first six months after the attack. The inversion of T

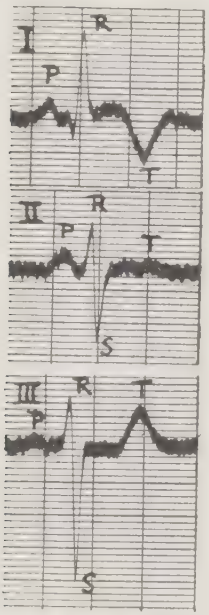

FIG. 19. Electrocardiogram taken three weeks after coronary thrombosis, showing the T1 type of curve. (D. E. Bedford.)

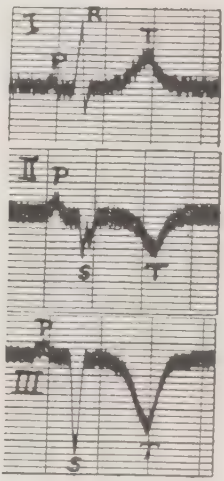

FIG. 20. Electrocardiogram taken one month after coronary thrombosis, showing the T3 type of curve. (D. E. Bedford.)

diminishes first in Lead II, then in Lead I or Lead III ; eventually T may become upright in all leads, but in some cases inversion of T persists almost unaltered. Evidence is accumulating that the T1 type of curve corresponds with infarction of the anterior wall or apex of the left ventricle (occlusion of the left anterior descending coronary) and the T3 type of curve with infarction of the posterior wall of the left ventricle (occlusion of the right coronary (Barnes and Whitten (14)).

Changes in QRS. Notching and widening of QRS are quite

frequent in curves after coronary thrombosis, though not in any way special to this condition. Low voltage of the QRS waves in all leads is also a common finding. A large Q wave in Lead III has been noticed in many cases of coronary thrombosis, and it is regarded as a suggestive sign of coronary disease, though it occurs in the chronic stages and occasionally in other forms of heart disease, or in displacement of the heart. It is thought to indicate damage to the posterior part of the interventricular

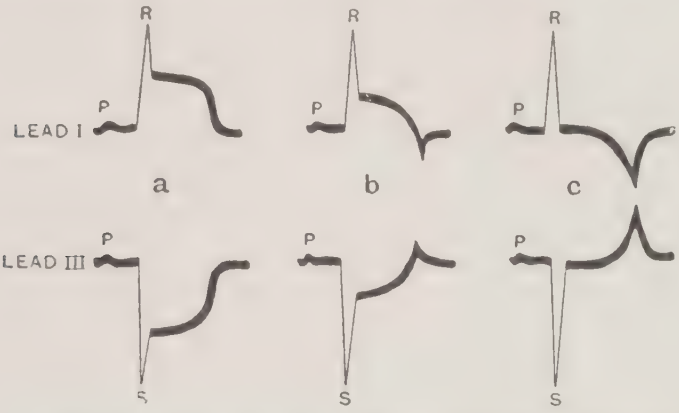

FIG. 21. Diagram illustrating the changes in the ventricular complexes in Leads I and III after coronary thrombosis. (a) R–T deviation; (b) T waves becoming evident; (c) R–T deviation disappeared. The T waves point in the opposite direction to the R–T deviation in each lead. (Parkinson and Bedford.)

septum (Pardee (15), Fernichel and Kugell (16)). The reader's attention is also directed to the diagram (see Fig. 21) which illustrates the electrocardiographic changes described above.

CHEST LEADS IN CORONARY DISEASE

Wolferth and Wood (17), in Pennsylvania in 1932, introduced chest leads in the electrocardiographic study of coronary occlusion, as "there are 'silent areas' in the heart where infarction may occur without producing a deviation of the S–T interval from the iso-electric line in any of the three conventional leads." The chest lead they employed was called Lead IV. The right arm electrode was placed anteriorly, "approximately at the cardiac level, just to the left of the mid-line." The left arm electrode was placed posteriorly at the same level, just to the left of the

mid-line. This lead has been largely adopted in America, and a normal Lead IV taken by this method shows negative **P** and **T** waves and a marked **Q** wave (see Fig. 22, *a*). If the position of the electrodes is reversed, so that the left arm electrode is placed on the front of the chest and the right arm electrode posteriorly, the normal curve shows a small upright **P** wave, a biphasic **QRS** complex, with equal **R** and **S** waves and an upright **T** wave (see Fig. 22, *b*). This method was used by Roth (18) in America in 1935. Roth also employed a right arm-chest lead, in which the right arm electrode remained on the right arm and the left arm electrode was applied to the fourth intercostal space 2 inches to the left of the mid-sternal line. With this lead the normal curve shows an upright and larger **P** wave, the **R** wave is larger than the **S** wave, and the **T** wave is upright (see Fig. 22, *c*). This lead has also been employed in England by Willcox and Lovibond (19) who urge its adoption as a standard.

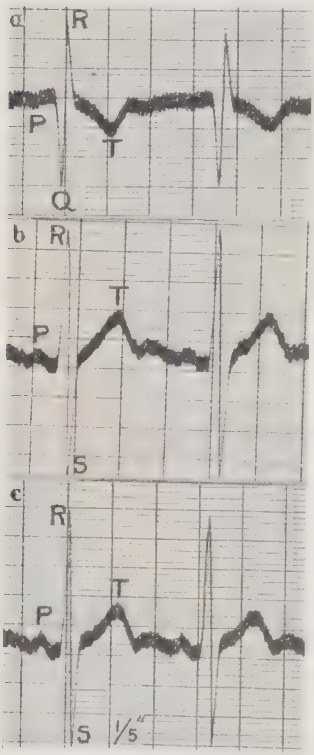

FIG. 22. Normal leads IV (a) right arm electrode anteriorly, left arm electrode posteriorly; (b) left arm electrode anteriorly, right arm electrode posteriorly; (c) left arm electrode anteriorly, right arm electrode on right arm.

In order to obtain comparable results it is very desirable that chest leads should be taken by the technique standardised by the joint recommendations of the Cardiac Society of Great Britain and Ireland and the American Heart Association (20). The præcordial electrode is placed on the extreme outer border of the apex beat, as determined by palpation. If the apex beat cannot be palpated the electrode is placed in the fifth left intercostal space just outside the left border of cardiac dullness or just outside the left mid-clavicular line. In our opinion the position of the apex in such cases can be better judged by auscultation. The variations of Lead IV are designated

as follows: Lead IV B; the præcordial electrode is paired with an electrode in the left interscapular region. Lead IV R; præcordial electrode and electrode on right arm. Lead IV L; præcordial electrode and electrode on left arm. Lead IV F; præcordial electrode and electrode on left leg.

In order to obtain standard polarity in chest leads, the lead switch is turned to Lead I. The left arm terminal is connected to the præcordial electrode. The right arm electrode is then placed in the left interscapular region for Lead IV B; it is kept on the right arm for Lead IV R; it is moved to the left arm for Lead IV L; and it is placed on the left leg for Lead IV F. With this arrangement relative positivity of the apical electrode is represented by an upward deflection, and relative negativity by a downward deflection. The normal curve with any of these variations of Lead IV will thus show an upright P and T wave.

The præcordial electrode should be circular, 2 to 3 cm. in

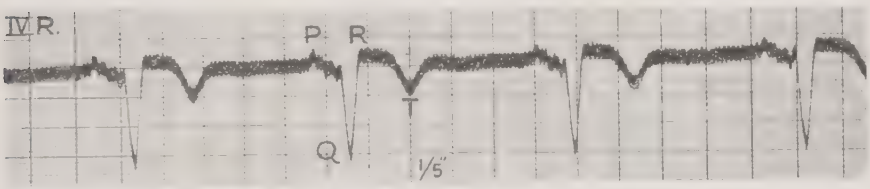

FIG. 23. Lead IV R in T_1 type of coronary thrombosis.

diameter, and made of German silver. The one we employ is held in position by suction, a little Cambridge jelly being placed on the skin under the electrode.

Results in Coronary Thrombosis. In the T_1 type of electrocardiogram (see Fig. 23) which corresponds with an acute anterior

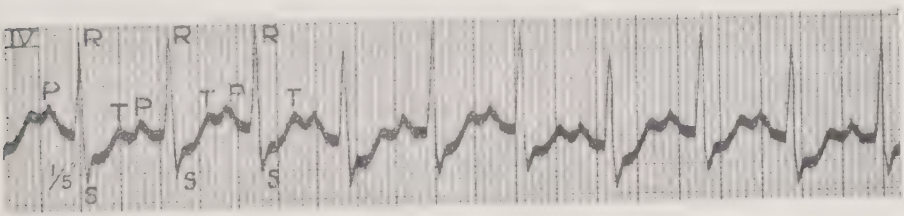

FIG. 24. Lead IV R in T_3 type of coronary thrombosis.

infarction, Lead IV shows an abnormal curve in the majority of cases. Thus, using the right arm chest-lead of Roth (Lead IV R), the typical findings are an elevation of the **R–T** segment, with an inverted **T** wave. The **R** wave is small or absent (see Fig. 23). The **T** wave may be huge, over 10 mm. In the T_3 type of electrocardiogram (see Fig. 24) which is met with in acute posterior infarction the changes in the fourth lead are not so constant, and not so characteristic as in the three conventional leads. The **R–T** segment is depressed, and the **T** wave is upright (see Fig. 24).

When acute anterior and posterior infarctions occur simultaneously, Wolferth and Wood (17) have shown that the **R–T** segment is raised in all the limb leads, especially in Lead II. In Lead IV R the **R–T** segment is also elevated. Wood and Wolferth (21) suggest that the huge **T** waves (over 10 mm. in amplitude) in Lead IV, whether positive or negative, indicate localised apical infarction.

Conclusions. Lead IV is of great value in suspected cases of coronary occlusion. In some cases it may show changes which are considered to be characteristic when the three conventional leads are inconclusive. Further, Willcox and Lovibond (19) showed that in 8 out of 62 cases of angina of effort Lead IV showed evidence of myocardial damage when the three conventional leads were normal. Such changes include absence of the **R** wave, notching of the **S** wave, deviation of the **R–T** segment from the iso-electric level and inversion of the **T** wave.

Some Cardio-therapeutic Measures

The Use of Digitalis. Digitalis still remains supreme amongst the drugs used in the treatment of heart disease. Withering (22), in 1785, was one of the first to draw attention to the value of digitalis in producing diuresis in certain cases of heart failure. Since then the indications for its use have become more clearly defined, and it is recognised that its most beneficial effects are obtained in cases of cardiac irregularity due to auricular fibrillation. It does not abolish the *circus* movement in the auricle, but slows the ventricle by increasing the degree of heart-block. It is also of value in auricular flutter, and, indeed, in heart failure from any cause. High blood pressure is not now

considered a contra-indication, as it has been shown that in therapeutic doses digitalis does not cause a rise of systolic pressure, although the pulse pressure may be increased as the result of a fall in the diastolic level in cases of cardiac failure. Whether or not it acts as a direct *cardiac tonic* and should be employed in disorders associated with sinus rhythm has been a matter of dispute. It is usually held that digitalis in such cases is a cardiac tonic and slows the whole heart by its action on the vagus, thus prolonging diastole, increasing the force of systole and improving the coronary circulation and so the nutrition of the cardiac muscle.

In heart failure of a severe degree large doses are usually required. On account of the danger of producing severe toxic effects, the total quantity necessary is generally administered in divided doses at several hours' interval, but the beneficial effect is thereby inevitably postponed. This delay is partially due to the fact that digitalis takes about six hours before it is absorbed from the stomach ; it is also eliminated slowly by the intestines and kidneys, and so cumulative results may occur.

Digitalis may be administered either in the form of a good tincture or as a tablet of the dried leaves. One cat unit tablet corresponds approximately with 1 ml. (m. 17) of the tincture or 0·1 g. (gr. 1·5) of tab. digitalis pulv. (B.P. Add.). In order to obtain results as quickly as possible in cases of severe heart failure the maximum amount of digitalis, which can safely be administered to an individual, should be given within twenty-four hours. This may be taken to be m. 17 of the tincture or gr. 1·5 of the dried leaf for every 10 lbs. of bodyweight. Thus, for a man weighing 10 stones the amount required is m. 240 of the tincture or gr. 21 of the dried leaf tablet. When the weight of the patient is not known accurately, as will often be the case, three-quarters of the above amounts should be the maximum given. Digitalis should not be administered more often than every six hours. Thus, in an urgent case, in which no digitalis has been taken within the last ten days, m. 60 of the tincture may be given six-hourly for 4 doses, and then the dose reduced to m. 30, if digitalisation has not yet been effected. As soon as this occurs the drug should be discontinued for a day or so and then m. 20 of the tincture or less may be required three times a day to allow for daily elimination and to keep the patient under the influence of the drug.

If digitalis has been administered within the last ten days the total calculated amount should be reduced to three-quarters if no signs of digitalis over-action are shown by the electrocardiogram, such as heart-block or premature ventricular systoles, and to one-half if they are present. In either case the total calculated amount is given in three equal doses at six-hourly intervals.

In conclusion, it may be said that massive doses should only be used in cases of heart failure of extreme urgency, and generally administration in smaller doses is the safest course to adopt.

The indications for discontinuing the drug are, according to Eggleston : Nausea or vomiting, unless this is due to venous congestion of the stomach, which is usually the case when it occurs shortly after the commencement of digitalis administration (Jensen (23)). The apex rate lower than 60. The occurrence of frequent ectopic systoles, heart-block, phasic arrhythmia or coupled beats. The latter are caused by a regular sequence of premature ventricular systoles following each normal ventricular contraction.

It is usually found that if the digitalis is going to relieve the heart failure diuresis takes place ; if there is no diuresis, beneficial results are not likely to ensue, and diminution in the secretion of the urine is an additional sign of digitalis toxæmia. The diuresis is a manifestation of the effect of digitalis in filling the arteries and emptying the veins, and is due to an increased renal circulation and relief of renal congestion. Diuresis does not follow the administration of digitalis, even in massive doses, unless œdema is present.

Digitalis should not be administered more frequently than at six-hourly intervals, as this is the time taken for its absorption from the stomach, and in cases of auricular fibrillation it has been shown by Canby Robinson (24) that an effect is produced in two to five hours after oral administration. Sufficient warning of any toxic effects, which may be produced by its administration, is thereby given.

Digoxin. Wokes (25), in 1929, showed that the leaves of the foxglove *Digitalis lanata* are physiologically nearly four times as potent as are those of *Digitalis purpurea*, the species used in medicine. Smith (26), in 1930, isolated a pure crystalline alcohol-

soluble glucoside, Digoxin, from the leaves of the *Digitalis lanata*. It possesses the advantage that it does not require biological standardisation. It is absorbed and excreted more quickly than is digitalis and can be given orally or intravenously. Intravenous injection is only advisable when an extremely rapid effect is required, or when the patient vomits anything taken by mouth. It must never be given intravenously if the patient has taken digitalis during the previous two weeks. In auricular fibrillation the average initial intravenous dose for an adult of 10 stones or over is 0·75 to 1 mg. A solution is obtainable in ampoules, each ampoule contains 0·5 mg. Digoxin in 1 ml. 70% alcohol. It must be diluted before use with 9 times the volume of freshly prepared sterile normal saline solution. The solution thus obtained is injected slowly into a vein, taking three to five minutes over the injection. The solution is very irritant and must not be injected outside the lumen of the vein. Wayne (27) has shown that the ventricular rate rapidly slows after such an injection, and that the maximum effect is obtained in one to two hours. Subsequently Digoxin is administered by mouth as described below.

For oral administration the initial dose is 1·5 mg. for patients weighing 10 stones or more, and 1 to 1·25 mg. for lighter patients. Digoxin must never be given by mouth if the patient has taken digitalis during the previous week. It is put up in " Tabloid " form, each containing 0·25 mg., to be taken in water, or in 30 ml. bottles containing 0·5 mg. in each millilitre of 80% alcohol. The solution is best given in chloroform water to disguise the bitter taste. After oral administration the ventricular rate begins to slow in about an hour and the maximum effect is obtained in about six hours. Six hours after the initial dose 0·25 mg. is given every six hours until the ventricular rate falls to 60 or 70. Subsequently a maintenance dose of 0·25 mg. twice a day may be required indefinitely.

Strophanthus. The action of strophanthus closely resembles that of digitalis. As an alternative method of producing a rapid effect upon the heart an intravenous injection of 1/200 to 1/100 gr. strophanthin may be given, provided that no digitalis has been administered within the last few days. The risk of producing toxic symptoms must always be borne in mind, as when the drug

is introduced by the venous route the safety-valve action of the stomach is of necessity eliminated.

Quinidine. This is a dextro-rotatory cinchona alkaloid, which was first used by Frey (28) in 1918 in cases of auricular fibrillation. Wenckebach (29) had previously, in 1914, obtained a temporary arrest of auricular fibrillation in one patient by giving 1 g. of quinine daily. Frey, in a series of 22 cases of fibrillation, obtained a restoration of normal rhythm in 50%. He gave the drug in doses of 0·4 g. (6 gr.) t.d.s. by mouth for three to eight days.

The administration of quinidine sulphate may be considered under the following headings :—

Selection of Case. Quinidine is of greatest value in cases of auricular fibrillation, especially when there is neither cardiac enlargement nor valvular disease (Cotton (30)). Best results are obtained in fibrillation of less than six months duration, and in those cases in which the symptoms increase with the onset of fibrillation. The infective group of cases is more suitable than the degenerative. The drug can be tried in paroxysmal auricular fibrillation, as digitalis does not usually have any beneficial effect here (Parkinson and Nicholl (31)). It may be of value in auricular flutter, the normal rhythm being restored direct, without an intermediate stage of fibrillation. It is at times successful in those cases of flutter which have been converted to fibrillation by the use of digitalis, but which do not return to the normal sinus rhythm on discontinuing the drug. It does not appear to have any beneficial effect on premature systoles.

Contra-indications. There are certain definite contra-indications which apply, no matter what the nature of the cardiac disorder may be. Thus quinidine must not be given if the heart is greatly enlarged. When there is congestive failure the patient should first be treated with rest, digitalis and diuretics until compensation is restored. Then, after a week's cessation from the use of digitalis, quinidine may be given if thought advisable. Heart-block is also a contra-indication. It must not be given if there is a history of recent embolism. It is considered inadvisable to administer quinidine if fibrillation has lasted for six months, or to use quinidine and digitalis simultaneously.

Regimen. The patient must be kept in bed, and preferably at absolute rest, during the initial week of the treatment. This

usually implies the necessity for skilled nurses. Attention must be paid to the bowels, and the diet should be such as will not give rise to flatulence.

Methods of Administration and Dosage. The drug is given by mouth in powder form in gelatine capsules. Probably the safest method is to give on the first day a test dose of 0·2 g. (3 gr.) to determine whether the patient has any idiosyncrasy to the drug. If no toxæmic symptoms appear, on the second day 0·4 g. (6 gr.) is given every three hours for four doses, and this is increased to five doses of 0·4 g. at three-hourly intervals on the third day. This dosage is then maintained until the end of the week, and if the normal rhythm has not been restored by this time the drug will probably not prove successful. The pulse should be taken before each dose is given, and if it is found to be regular the drug should be discontinued, at any rate temporarily, until a tracing has been taken. Further indications for discontinuing the administration of quinidine are a slowing of the auricular rate to about 250 per minute, or an increase of the ventricular rate to over 160. In the former case there is a danger of the ventricle following directly the auricular rate. The exact rate of the auricle and ventricle are best determined by an electrocardiogram

Hay (32) recommends that quinidine should be given every two hours by day in ten equal doses, as its action soon passes off. Starting with a test dose of 0·2 g. (3 gr.) on the first day, 2 g. (30 gr.) are given on the second day in ten doses of 0·2 g. each. If necessary, the dose may be increased to 3 g. (45 gr.) a day in ten doses of 0·3 g. (4·5 gr.). The smallest beneficial dose appears to be one of 5 gr., which restored the normal rhythm in a case of auricular fibrillation of recent origin, under our care, which was associated with a toxic goitre. As much as a total of 375 gr. has, however, been required before a satisfactory result was obtained, and it may be necessary to continue indefinitely with small doses of 5 gr. daily, especially if a relapse occurs on omitting the drug.

Symptoms of Toxæmia and Dangers. Frey (28), in 1918, drew attention to the possibility of quinidine idiosyncrasy, resulting in such symptoms as respiratory failure and cerebral paralysis. Benjamin and V. Kapff (33), in 1921, recorded instances of embolus occurring during the administration of quinidine. This

is especially liable to follow restoration of normal rhythm, and is, in all probability, due to the detachment of a clot from the wall of the auricle. Embolus formation is not necessarily fatal. The onset of ventricular fibrillation may cause sudden death.

Headache is nearly always experienced while quinidine is being taken. Other toxic symptoms include sweating, nausea, vomiting, diarrhœa, abdominal pain and a scarlatiniform rash. Dimness of vision, or the occurrence of frequent ectopic ventricular beats, is an indication for the immediate discontinuance of the drug.

Results of Quinidine Administration. Campbell and Gordon (34) found that quinidine restored normal rhythm in 64% of a series of 135 cases of auricular fibrillation. In 30% of these, however, fibrillation recurred after an average period of two years, but in the other 34% the normal rhythm was still maintained after an average period of nearly four years.

The Action of the Drug. The effect of quinidine upon heart muscle can be summarised thus: The auricular rate is slowed. The rate of conduction in the auricle, ventricle and A–V junctional tissues is reduced. The absolute refractory period of auricular muscle is prolonged.

Quinidine has also some effect upon the vagus nerve, causing a partial paralysis which tends to increase the ventricular rate.

The electrocardiographic changes which may occur during quinidine administration are, according to Hay (32): Slowing of auricular rate; the onset of auricular flutter and 2 : 1 heart-block; increase of ventricular rate; appearance of ectopic ventricular beats; restoration of normal rhythm in 50% of cases of fibrillation.

The Value of the Drug. The administration of quinidine has very definite dangers, and the cases in which it is worth a trial require careful selection, and very close watching during the treatment. Even if normal rhythm is established, the general condition of the patient is not always improved, while the underlying myocardial degeneration still remains.

Mercurial Diuretics and Ammonium Chloride. These drugs include mersalyl, Neptal, Mercupurin, Novurit, and Esidrone. They are of great value in the treatment of congestive failure (right ventricular failure), and in acute œdema of the lungs (left

ventricular failure). Mersalyl is an organic mercurial compound which was reported on clinically by Brunn (35) and by Bernheim (36) in 1924. It contains 36% of mercury, and is put up in 10% solution in 1 ml. and 2 ml. ampoules. Mersalyl has now replaced novasurol which was introduced by Saxl and Heilig (37) in 1920. Novasurol has been abandoned owing to its toxic effects, whereas mersalyl is not usually toxic. Andrews (38) reported a case of heart failure, without renal disease, in which the first intravenous injection of 0·5 ml. of mersalyl resulted in headache, epileptiform convulsions and unconsciousness, but the patient, a woman aged twenty-two, did not die. In the case described by Higgins (39) there was hypertensive heart disease with congestive failure. About 10 doses of mersalyl or Mercupurin had been given, when an intramuscular injection of 1 ml. of Mercupurin was followed an hour later by a severe chill, fever, dyspnœa, cyanosis, fall in blood pressure and prostration. Death seemed imminent. A month later the patient died and the kidneys showed some mild arteriolosclerosis, with very slight necrosis and calcification of the tubules, probably due to mercury. Volini et al. (40) reported 3 cases of very sudden death following the intravenous injection of mercurial diuretics, 1 from Mercupurin and 2 from Esidrone. In 2 of the cases electrocardiograms showed the development of ventricular fibrillation. Evans and Perry (41) have also published 6 cases of immediate death due to the same cause, and suggest that it may be advisable to give mercurial diuretics intramuscularly to patients with a low plasma protein. In other cases cramps may develop in the legs due to depletion of chlorides, and these can be relieved by the administration of sodium chloride. Mercurial diuretics should not be given in cases of acute nephritis, in lipoid nephrosis, or if the urine contains some granular or blood casts, or if there is hæmaturia. A test dose of 0·5 ml. mersalyl is first injected intramuscularly, and if there is no intolerance, as shown by hæmaturia, diarrhœa or irritation of the skin, a course of intravenous injections is begun. In order to render the urine acid, ammonium chloride in doses of gr. 30, is given by mouth every six hours. The ammonium chloride can be administered as a mixture: Ammon. chlor. gr. 30, ext. glycyrrhiz. liq. m. 20, sp. chlorof. m. 7, aquam ad 1 oz., or in the form of four 0·5 gram "stearettes." On the third day of the

administration of the ammonium chloride, 1 ml. of mersalyl diluted with 4 ml. of sterile normal saline is injected slowly into a vein in the arm. The arm is then raised to prevent venous thrombosis. The ammonium chloride is administered daily. On the fifth and ninth day 2 ml. of mersalyl, diluted with 8 ml. of sterile normal saline are injected intravenously. If a good diuretic response is obtained, such as 90 to 180 oz. of urine, the injections of mersalyl should be repeated at intervals of a week, the ammonium chloride being given by mouth for two days before, the day of, and the day after the injection, *i.e.*, for four days each week. The total fluid intake during the twenty-four hours must be restricted to 35 oz. or less, and full digitalisation should be maintained to control the rate of the heart. The fluid intake and output should be recorded each twenty-four hours, and the patient weighed at first daily and later weekly. The degree of dehydration is reflected in the loss of weight. No salt should be used in the cooking of the food, and none added when it is eaten. In some cases we have found that the administration of ammonium chloride alone will produce a good diuretic response.

In 1933, Hermann, Schwab, Stone and Marr (42) suggested the use of a combined mercurial diuretic and a purine base, on the supposition that the purine diuretic increases glomerular filtration and the heavy metal diminishes tubular re-absorption. Such a preparation is available in the form of Novurit, a mercurial theophylline compound, which is injected in doses of 1 or 2 ml., preferably intravenously ; if this is difficult, intramuscularly, but never subcutaneously, or sloughing may ensue. If given intramuscularly it should be injected into the upper and outer quadrant of the gluteal region. The ammonium chloride should be administered by mouth, as in the case of the injection of mersalyl. The diuretic response is usually extremely satisfactory, and in some cases exceeds that obtained with mersalyl. Parkinson and Thomson (43) recorded their experiences with the use of Novurit rectal suppositories, each containing 0·5 g. Novurit in a cocoa-butter basis. In our experience these suppositories are not always satisfactory ; in some instances proctitis has ensued, in others there has been no diuretic response, although a good effect has been subsequently obtained by the intravenous injection of the drug.

It is important that the weekly injections of mersalyl or

Novurit, with the corresponding administration of ammonium chloride, should be continued for an indefinite period providing a good diuresis ensues, and that the patient does not become unduly dehydrated. We have patients who have received this treatment for one to two years, and who are still continuing with it, thereby being enabled to maintain a fair degree of health and activity. In other cases we have noticed that dehydration appears excessive after a prolonged course—the patient feeling weak and ill after each injection. Here the injections should be discontinued, and if required again later they should be given at longer intervals. It should be remembered that as much as 8 to 10 pints of excess fluid may be retained in the body tissues without pitting œdema being apparent. Such a condition may be termed latent œdema, and is associated with pulmonary congestion and œdema of the lungs, and with an engorged liver. It is much relieved by the administration of mersalyl or Novurit.

Carbachol. Dale (44) has brought forward evidence to show that when parasympathetic nerves are stimulated, acetyl-choline is formed at the nerve endings, which transmits the nerve impulse to the effector organ. The acetyl-choline is rapidly hydrolysed and rendered inert by choline esterase. Acetyl-choline is an unstable substance, and for clinical use carbacholum B.P. Add. (Doryl) is more satisfactory. Carbachol has been used to slow the rate of the heart in paroxysmal tachycardia of supraventricular origin, without congestive failure. It can be given by mouth in tablets containing 2 mg. The usual dose is ½ to 2 tablets one to three times a day. It may also be given by subcutaneous or intramuscular injection of 1 ml. of a solution containing 0·25 mg. carbachol. It should never be given intravenously. An attack of paroxysmal tachycardia may sometimes be averted at the earliest premonitory system by taking 1 carbachol tablet (2 mg.) by mouth, and in some cases the daily administration of ½ to 2 tablets by mouth has appeared to prevent the occurrence of further attacks.

Thiocyanates and the Treatment of Hypertension

Potassium and sodium thiocyanate were originally recommended for the treatment of essential hypertension by Pauli (45) in 1903. The mode of action is obscure, but it has been suggested that they cause a relaxation of smooth muscle. They are readily absorbed

from the gastro-intestinal tract and are distributed throughout body fluids in the same way as chlorides and bromides ; they do not penetrate into the cells. They are excreted mainly through the kidneys, but this excretion can vary as much as 400% in different individuals.

Thiocyanates offer one of the few means of treatment of hypertension, but their use was limited, owing to toxic effects, until the dosage was controlled and rendered more safe by serum estimations. Barker and his colleagues (46, 47 and 48), who have treated many patients successfully over a number of years, emphasise the importance of frequent determinations of serum levels of thiocyanates while treatment is in progress. They consider a concentration of 8 to 12 mg. per 100 ml. to be a safe level in the majority of cases. They recommend beginning the treatment by giving 0·3 g. per day and making blood cyanate determinations every week for four to six weeks, and subsequently every month. It is readily soluble, 0·1 g. in ½ oz. of water being given three times a day, or it may be prescribed as Elixir Sodium Thiocyanate (gr. 20 in fl. oz. 1), one teaspoonful twice a day. Enteric-sealed tablets, containing 0·2 g., or 0·065 g. of potassium thiocyanate are also sometimes given. If the blood pressure drops sharply, or if symptoms of fatigue or depression are marked, the dose can be decreased. If no blood pressure change or toxic symptoms occur after three to four weeks, and the blood concentration has not reached 6 mg. per 100 ml., the dose may be doubled. Barker and his colleagues, who reported on 246 cases, found that the systolic pressure may drop during the first two to four weeks, whereas the diastolic pressure may not be reduced for three to four months. The best clinical results were obtained with patients whose erythrocyte count was high and sedimentation rate low, and whose blood pressure was of the fluctuant type. Out of 246 patients, 76% showed some improvement. There were no toxic deaths, but 18 patients showed toxic manifestions of one kind or another. Another series of 50 cases treated over eleven years, was reported by Kurtz, Shapiro and Selby Mills (49). These authors followed Barker's method of laboratory control. Subjective improvement was very definite in 63% of cases and fair in 17%. Satisfactory reduction of blood pressure was obtained in 78% of cases. There were no deaths that could be attributed

directly or indirectly to thiocyanate. Fischmann (50) treated 40 cases with thiocyanate, 25 proving successful and 15 being failures. Of the successful cases, 3 showed no fall in blood pressure but improved symptomatically, especially as regards headache. Of the 15 failures the blood pressure fell in 6 cases without subjective improvement.

To set against these encouraging results, there have been some reports of fatalities and toxic effects following the use of thiocyanates. Foulger and Rose (51) in 1943 and Potter (52) in 1944 reported cases of acute goitre due to thiocyanate therapy. Other toxic effects include exfoliative dermatitis, weakness, dizziness, anorexia, thrombo-phlebitis, and hæmorrhagic encephalomalacia. The importance of a strict control of dosage based on weekly and later monthly serum determinations, and a careful watch for untoward symptoms cannot be over-emphasised. Further, thiocyanate should never be given if there is any evidence of renal failure. In our experience it may not lower the blood pressure, or the fall produced is only temporary.

The Circulation Time

Various methods have been employed to estimate the circulation time in man. Bornstein (53) in 1912 made the patient breathe a mixture of air containing 5 to 7% CO_2. The time taken for the arterial blood to carry an increased amount of CO_2 to the respiratory centre was shown by an increased depth in the breathing. Since then various substances have been injected intravenously, such as fluorescin, histamine, calcium chloride, saccharin, ether, sodium dehydrocholate, calcium gluconate, and magnesium sulphate. Tarr, Oppenheimer and Sager (54) in 1933 gave a historical account of the methods which have been used, and reported their experiences with sodium dehydrocholate (Decholin Sodium). This measures the arm-to-tongue circulation time. The test is carried out before breakfast, with the patient lying as flat as possible, the right arm being supported at the level of the auricles. Five ml. of a 20% solution of Decholin are injected through a wide-bore needle into an antecubital vein, taking one to two seconds over the injection. The patient is told to raise his left hand directly he perceives a bitter taste under the tongue. The time is taken with a stop watch from the beginning

of the injection of the Decholin to the signal that it has reached the tongue. The average normal time is thirteen seconds (extremes ten to sixteen seconds). Occasionally the patient complains of nausea after the injection, or of pain in the region of the gall-bladder. The circulation time is not affected by the weight, height, pulse rate or blood pressure of the patient. It is prolonged in congestive heart failure in the majority of cases, to between twenty and forty-seven seconds. The circulation time is also prolonged in myxœdema, and polycythæmia, but shortened in fevers, anæmia, and hyperthyroidism, when the latter is not complicated by heart failure. Bernstein and Simkins (55) in 1939 used magnesium sulphate to determine the arm-to-tongue circulation time. Six ml. of a 10% solution of magnesium sulphate are injected intravenously into an antecubital vein, and when it reaches the mouth the patient experiences a transient hot sensation in the pharynx and tongue, which spreads rapidly to the face, hands, perineum and legs. It is advisable to have at hand an ampoule of 10% calcium gluconate for immediate intravenous injection should an undue magnesium sulphate reaction occur. With this method Bernstein and Simkins found the average arm-to-tongue circulation time 12·9 seconds (extremes 7 to 17·8 seconds). They classified cases of heart failure clinically into groups: (1) well-compensated; (2) with dyspnœa on exertion; (3) with dyspnœa and cyanosis; (4) with additional pulmonary congestion; (5) with ascites or œdema. In the majority of cases the circulation time was prolonged proportionately to the degree of heart failure, up to about thirty seconds. Lesions such as mitral stenosis or auricular fibrillation, if not accompanied by decompensation, did not affect the circulation time. The authors state, however, that the circulation time is not "the infallible diagnostic tool" it was once supposed to be, and it is not really uncommon to find a normal circulation time in a markedly decompensated patient. However, serial readings are of value, as the circulation time in an individual varies according to changes in his condition, being prolonged when he is worse and *vice versâ*. They also found it of value in determining whether there is, or is not, cardiac failure in the presence of emphysema, *i.e.*, whether the dyspnœa is predominantly pulmonary or cardiac. If the circulation rate is rapid, then the heart is well compensated. In

7 cases of bronchial asthma the arm-to-tongue circulation time was found to be shortened. The ether circulation (arm-to-lung time) is measured by injecting into an antecubital vein 5 m. of ether well shaken with 5 m. of normal saline, using a tuberculin syringe and a large needle. The time is recorded from the moment of injection to the moment when the patient or the observer smells the ether in the breath. The average normal time is 5·7 seconds (extremes 2·6 to 10 seconds). The arm-to-tongue time measures roughly the circulation rate as a whole, the arm-to-lung time indicates the functional capacity of the right ventricle. In right-sided failure, therefore, the ether time is prolonged. The lung-to-tongue time (the Decholin or the magnesium sulphate time minus the ether time) is a test for the functional capacity of the left ventricle. Thus Hitzig (56) showed that in isolated failure of the left ventricle the arm-to-tongue circulation time is almost always prolonged, sometimes up to three times its normal value, but the arm-to-lung time may be simultaneously normal. Occasionally, however, with severe left-sided failure the arm-to-tongue circulation time is normal, possibly owing to a very powerfully acting right ventricle. Cottrell and Cuddie (57) have used the Decholin method to investigate the circulation time in 21 cases of bronchial asthma, and found it to be normal both during and between the attacks.

Conclusion. In our experience, using the Decholin method, the arm-to-tongue circulation time has proved of value in distinguishing between cardiac and pulmonary dyspnœa, especially in cases of emphysema or bronchial asthma complicated by auricular fibrillation, myocardial degeneration, or a valvular lesion.

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CHAPTER IX

THE LUNGS

Primary Atypical Pneumonia

Owing perhaps to the greater facilities for, and importance attached to, radiographic examinations of the lungs, attention has been increasingly directed during recent years to certain catarrhal pulmonary conditions, usually of a benign character, in which the X-ray films show soft shadows in the lung fields. The term primary atypical pneumonia has been used to cover a group of allied diseases, such as virus pneumonia, pneumonitis, influenzal pneumonia, broncho-pneumonia of unusual or undetermined ætiology, acute diffuse bronchiolitis, acute bronchiolitis with atelectasis, and atypical pneumonia with leucopenia. When we have excluded conditions of which the cause is thought to be known, such as influenza due to Pfeiffer's bacillus or to the viruses A, B or X associated with staphylococci or streptococci, the pneumonic consolidation of psittacosis, Rickettsia infections such as the Q fever of Australia, American Q fever and ornithosis, we are left with a group of cases in which the cause is unknown, but is probably a virus, and which may be designated as primary atypical pneumonia, virus pneumonia, or virus pneumonitis.

Primary atypical pneumonia tends to appear in small epidemics and probably also exists endemically. It is not highly contagious but may infect doctors and nurses looking after cases in hospital wards. Young adults are chiefly affected and there is no particular seasonal incidence. The disease has not been transmitted to animals, but Bragg (1) in 1945 successfully conveyed it to human volunteers by spraying the nose and throat with a bacterium-free filtrate obtained from the throat washings and sputum collected from patients suffering from atypical pneumonia. Thus 3 out of 12 men receiving this inoculum developed the disease 12 to 14 days later, and cold agglutinins for group O human red cells were found in their serum in titres varying from 1/128 to 1/1,024, 20 to 27 days after infection.

The mortality rate of the disease is low, about 0·2%, and few

observations have been published on the post-mortem findings. Longcope (2) observed areas of deep-red moist solidification in the lungs, with purulent material in the bronchi. The alveoli in the affected areas were filled with coagulated serum containing red corpuscles and mononuclear cells, polymorphonuclears being rarely seen. Campbell et al. (3) consider that the primary process is a peribronchitis, peribronchiolitis, bronchitis and bronchiolitis, with swelling of the bronchial epithelium and exudation into the bronchi and bronchioles, resulting in œdema, absorption of the contained air and localised areas of collapse. The alveolar walls become thickened and the alveoli contain mononuclears. Areas of encephalitis were also seen. Perrone and Wright (4) reported a fatal case of primary atypical pneumonia complicated by encephalitis, the patient dying on the fourteenth day of the illness. Foci of increased cellularity were seen in the cerebrum, cerebellum, basal ganglia and paraventricular tissue. The cells forming these foci were astrocytes and microcytes. There were also areas of lymphocytic perivascular infiltration in the meninges.

Clinical and Laboratory Findings. The incubation period varies from two to twenty-one days or longer. The onset is frequently insidious with malaise, severe headache, a racking dry cough and muscular pains in the limbs and abdomen. Anorexia may be a marked feature, and at times the patient complains of pain in the chest and dyspnœa ; or the illness may begin with an upper respiratory tract catarrh. Some cases are apyrexial, the patient having a persistent cough with catarrhal symptoms, and the nature of the disease is only recognised when the lungs are examined radiographically. The temperature usually rises to 100° or 103° F., and after remaining at about this level for eight to ten days, falls by lysis, although secondary rises of temperature are sometimes met with.

The sputum is usually mucoid and at times contains a few streaks of blood, or small hæmoptyses of a teaspoonful or so occur, but the expectoration is never "rusty" or of a "prune juice" appearance. It contains no predominating or characteristic organisms, but mononuclear cells may be seen. The physical signs in the lungs are indefinite and variable, small areas of collapse, indicated by slight dullness and weak air entry, may appear from day to day. The most characteristic finding

is the presence of showers of sticky râles, heard especially after cough, over the affected portions of the lungs. In some instances there is dry pleurisy and rarely a pleural effusion develops. The pulse rate is often lower than would be expected from the temperature. In more severe cases there is marked dyspnœa and cyanosis, the temperature is high and irregular, the patient being severely prostrated. The white cell count is usually normal but there may be a slight leucopenia. The presence of a leucocytosis would indicate the advisability of reconsidering the diagnosis. The blood Wassermann reaction may be positive for a short period, and during the acute stage of the illness the sedimentation rate of the red cells is increased to between 20 and 40 mm.% plasma at one hour.

Peterson, Ham and Finland (5) described the presence of cold agglutinins in high titre in the serum of patients suffering from primary atypical pneumonia, and this observation has been confirmed by Horstmann and Tatlock (6), and others. In the majority of cases the reaction is positive between the second and fourth weeks of the illness. Dilutions of the patient's serum are made from 1/4 to 1/4,096 in 1 ml. volume, and to each tube is added 0·1 ml. of a 2% suspension of washed group O human erythrocytes. The tubes are kept at 0° C. overnight, and readings made immediately on removing them from the ice box, and again after they have stood at room temperature for several hours, to see whether the reaction is reversible at room temperature, and so indicative of the presence of a true cold agglutinin. The test is not entirely specific as it has been observed in African trypanosomiasis.

Although there are no typical X-ray findings, the diagnosis cannot be made in the absence of abnormal shadows in the skiagram of the lungs. There is often a marked discrepancy between the extent of the clinical and radiological findings, the latter being more extensive ; further the shadows in the film frequently persist for some time after convalescence is established and the patient is afebrile. Patchy opacities may be seen, somewhat resembling those cast by areas of bronchopneumonia. They are often irregular in shape and vague in outline and may have a hazy or ground-glass appearance, migrating in position from day to day. At times the affected areas present a reticulated appearance. Camp-

bell et al. (3) found that 81% of the lesions were situated at the base, each side being equally affected. Drew et al. (7) observed that the costo-phrenic angle, especially on the left side, is a common site for the shadow, and, owing to areas of atelectasis, the diaphragm may be raised on one side and the mediastinum correspondingly displaced. Complications are rare, the violent cough has been thought to give rise to multiple fracture of ribs, and encephalitis is a dread development. Venous thrombosis in the legs, and polyarthritis have been occasionally described.

Cases may be mistaken for pulmonary tuberculosis, especially when the X-ray shadows are situated near the apex, and in other instances an erroneous diagnosis of primary atypical pneumonia has been made on radiographic evidence, when the patient was in reality suffering from lung abscess or bronchial carcinoma. At times the prolonged continuous type of fever with a relatively slow pulse rate and low white cells count suggests enteric fever. In influenza no abnormal shadows are likely to be seen on X-ray examination of the lungs. Difficulty may also arise with cases of pneumonia which have been treated with sulphonamides, when the signs in the lungs persist, there is no leucocytosis, and the X-ray shadow has a reticulated appearance. Further, an area of basal bronchiectasis with surrounding inflammatory changes may lead to diagnostic difficulty. The lesions of primary atypical pneumonia do not give rise to subsequent pulmonary fibrosis.

Treatment. It is most important to keep the patient in bed until the temperature has been normal for a week, and he should not resume work until the sedimentation rate of the red cells is normal and the X-ray shadows have disappeared. Penicillin and the sulphonamides are useless, and the latter may be dangerous giving rise to a hæmolytic crisis. Promising results, however, have been obtained by the use of convalescent serum. Steam inhalations may relieve the distressing cough, but it is wise to avoid the use of opiates, except in small doses. For severe cyanosis and dyspnœa oxygen should be administered by nasal catheter or the B.L.B. mask.

Blast Injuries of the Lungs

Experimental Work. It was noted during the 1914–18 war that soldiers might be killed by shell explosion without any external

signs of injury. Attention was again drawn to the subject by Barcroft (8) in 1939 in his experiments concerned with the protection against blast afforded by the Anderson shelter. The effects on goats of blast produced by the explosion of a 500 lb. bomb were investigated. If the goat was in the open, and within 15 feet of the explosion, hæmorrhagic lesions were produced in the lungs. Zuckerman (9) in 1940 investigated the effects of blast, produced by the detonation of high-explosive, on small animals such as the rat, mouse, rabbit, cat, monkey, etc. When the explosion occurs there is first a wave of high pressure, which, in open spaces, may reach twelve times that of the atmosphere, within a radius of a few feet. The pressure at a comparatively small distance is, however, considerably less, so that at 100 feet it is only slightly above normal. The positive pressure wave is followed by a negative pressure or suction wave of considerably smaller degree, and both waves only last for a fraction of a second. Hæmorrhages of varying extent were found in the internal organs, especially in the lungs, but even when death resulted there were no signs of external injury. There was often blood in the upper respiratory passages. The most vulnerable portions of the lungs are the anterior and inferior borders, and the costal and mediastinal surfaces. These are the sites where the pressure effects of the compressed chest wall are the greatest. The surface hæmorrhages often followed the lines of the ribs. In the lungs the alveoli and smallest bronchi were filled with blood, the alveolar capillaries being torn. Fat emboli were not found. Further, the liver may be bruised or torn, hæmorrhages may be seen in other abdominal organs, especially in the intestinal wall, and extradural hæmorrhages were frequently seen around the thoracic spinal nerve roots. When the blast pressure was high the ear drums were ruptured. Zuckerman concluded that the pulmonary lesions resulted not from air being forced under high pressure down the trachea, nor from air being sucked out of the lungs, but from the direct pressure of the body wall upon the lungs. The pulmonary lesions resulting from blast resemble those due to falls or to crush injuries of the chest, met with in civil life, to which attention had been drawn by Fallon (10), Ross (11), and Osborn (12). Zuckerman et al. (13), from further experiments on rabbits exposed to blast, concluded that immediate death un-

associated with external injury is probably due to shock following internal injury; further, the larger air-passages may be occluded with blood clot. Delayed death is usually due to œdema of the lungs, and more rarely to intraperitoneal hæmorrhage.

Clinical and Pathological Findings. The effects of blast injuries in man have been well described by O'Reilly and Gloyne (14). Shock is an important feature, the patient being pale and cyanosed, and the pulse frequent and feeble. There is also distressed breathing, the chest bulging especially at the lower costal margin, as described by Dean et al. (15), and being partially fixed in a position of inspiration. Pain may be felt in the chest, either centrally, or near the surface, or in the abdomen. In some cases the condition suggests an acute abdominal lesion. The sputum may be stained with blood, or the patient may cough up an ounce or so of fresh blood. Restlessness is a constant feature, and it may be severe, depending upon the degree of blast damage. Rupture of the ear drums was frequently met with, and this may be followed by otorrhœa. The breath sounds over the lungs were weak, and coarse râles were scattered through the lung fields. In some cases the temperature rose to about 104° F., with signs of a basal broncho-pneumonia, due to a hæmorrhagic alveolar exudation. Radiographic examination revealed scattered areas of mottling, which disappeared in a few days in favourable cases. At autopsy, hæmorrhages were found in the intercostal muscles, fluid blood in the pleural sacs, hæmorrhages in the lungs, and an effusion of blood into the mediastinum. Spontaneous pneumothorax may occur. Fat embolism of the lungs was not found, nor were the hæmorrhagic lesions most marked in the phrenico-costal sinuses, as in the cases recorded by Osborn (12).

Treatment. This resolves itself into careful nursing, the administration of morphin. sulph. in doses up to gr. $\frac{1}{3}$ followed, if necessary, by gr. $\frac{1}{6}$ to control restlessness, the use of electric cradles and hot bottles for shock, and the continuous administration of oxygen by a nasal catheter or the B.L.B. (Boothby, Lovelace and Bulbulian) mask. In some cases venesection is helpful, but whole blood transfusion, and, probably also plasma transfusion, are contra-indicated owing to the risk of further pulmonary embarrassment. Anæsthetics are inadvisable, but, if

necessary, should be restricted to local administration or to Pentothal Sodium.

The Vital Capacity

The vital capacity is a measurement of the maximum volume of air which can be expired after a full inspiration.

In our experiments we have determined the recorded vital capacity and compared it with the estimated normal vital capacity as judged by the mean of five standards : body weight, standing height, trunk height, chest circumference and surface area. We have employed the Hutchinson spirometer. The vital capacities

Vital Capacity Case Sheet

Name, P.G.F. *Date*, Feb. 27th, 1933. *Sex*, M. *Age* 37.
Occupation, Petrol Depot Inspector.

Measurements.	Vital Capacity Calculated by Myers' Tables.	% Deviation.
Body weight, 121 lb. . .	59%	−41
Standing height, 5 ft. 7¼ in. .	52%	−48
Trunk height, 34½ in. . .	56%	−44
Chest circumference, 30½ in. .	64%	−36
Surface area, 1·60 sq. m. .	55%	−45
	5	−214

Vital capacity recorded = 2,200 ml. = −43%
Therefore normal = 3,860 ml.
Sedimentation Test. One hour reading, 7·8 cm. red cells.
2·2 cm. plasma.

are recorded with the individual sitting, and it is found that the vital capacity is about 5% lower when recorded in the recumbent position. These figures are worked out from Myers' (16) tables, and the results are charted as shown in the example above.

We have carried out vital capacity observations on these lines on a series of persons in health and disease.

(*a*) *Athletes.* An Oxford University rowing crew in the latter

days of training for the Boat Race. Average vital capacity, 4,856 ml. Maximum, 5,750 ml. Minimum, 4,000 ml.

Flack (17) gives a figure of 4,000 as the average for a fit flying officer.

(*b*) *Pulmonary Tuberculosis.* One hundred cases were examined. The activity of the disease was determined by temperature records, sputum and sedimentation tests. The extent of disease was determined by clinical signs and by X-ray examination.

Results.

Active, T.B. +.	63 cases.	Average vital capacity			— 36%
„ T.B. —.	18 „	„	„	„	— 30%
Non-active.	13 „	„	„	„	— 16%
Suspicious.	6 „	„	„	„	— 34%

The relationship between the vital capacity in active cases of pulmonary tuberculosis and the sedimentation rate of the red cells of the blood in a series of 60 cases is shown in the accompanying graph (see Fig. 25). It will be seen that the curve indicates a close relationship, when an allowance of a $\pm$ 10% variation is made for the normal vital capacity, and also for the fact that some of the cases of pulmonary tuberculosis are suffering from bronchitis or emphysema which of themselves lower the vital capacity. We have also plotted the relationship between the extent of lung involved and the vital capacity. For this purpose we have not adopted the Turban-Gerhardt anatomical classification of tuberculosis, for the reason that any case in which there is a cavity present is classified as Stage III., the most advanced stage. As there may only be one cavity near an apex and the remainder of the lung normal, it obviously does not form a satisfactory classification when grouping cases according to the actual lung territory involved by the tuberculous process. We therefore classified our cases in six groups according to whether 1/3, 2/3 or the whole of one or both lungs is involved.

The results of the graph show that there is no relationship between the vital capacity and the extent of lung involved.

The vital capacity recorded depends upon the volume of the lungs and the expiratory force. As in pulmonary tuberculosis the vital capacity is affected chiefly by the activity rather than

by the extent of lung involved, it would appear that the expiratory muscle power is lowered by the toxæmia. The results we obtained, however, using the mercury manometer test as an indication of the expiratory power, suggest that the expiratory power is not lowered more in active than in non-active cases. It is possible therefore that there is another factor concerned with the vital

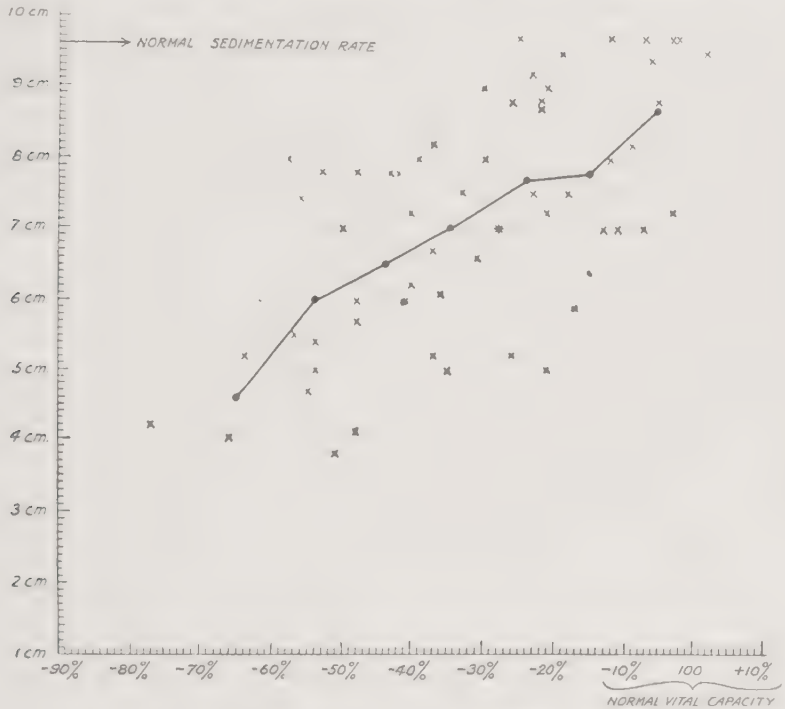


FIG. 25. Chart showing relationship between the vital capacity in Pulmonary Tuberculosis and the activity of the disease, as judged by the sedimentation rate of the red cells of the blood in sixty cases. The curve is plotted along points which are means of the individual readings. In two instances, shown by the stars, the readings of two cases are identical.

capacity, such as a diminished entrance of air into the bronchioles or alveoli, which may be influenced by the toxæmia.

Conclusions. We have determined the vital capacity in healthy individuals and in many cases of diseases of the lungs. The vital capacity is lowered in all diseases of the lungs and pleura, with the possible exception of adherent pleura. If the vital capacity reading is normal, it is very unlikely that disease of the lungs is present. Exceptions occur from time to time in

athletes, or in those who have in the past been athletes, when a normal or even plus vital capacity is obtained, although the lungs are affected. The degree of diminution of the vital capacity in pulmonary tuberculosis depends upon the activity of the disease rather than upon the extent of the lesion, and serial readings therefore afford an indication of the progress of the case. With a very low vital capacity, below 25% of the normal, extensive surgical operations such as thoracoplasty are contra-indicated.

Artificial Pneumothorax

Historical. Carson (18), of Liverpool, in 1822, first suggested that air might be admitted into the pleural cavity as a therapeutic measure in certain diseases of the lungs. Cayley (19), in 1885, at the Middlesex Hospital, London, was responsible for the establishment of a pneumothorax by pleural incision in a case of intractable hæmoptysis. Forlanini (20), in Italy, must undoubtedly be regarded as the originator of the modern pneumothorax treatment by puncture for pulmonary tuberculosis, his work dating back to 1882. Lillingston (21), in 1910, was one of the first to adopt this method in England.

The Indications

Collapse of the lung, by the introduction of air into the pleural cavity, is of value chiefly in the treatment of pulmonary tuberculosis, less often for bronchiectasis, localised abscess of lung and interlobar empyema. It has also been used for the relief of pain in dry pleurisy, for pneumonia, massive collapse of the lungs, and for broncho-pneumonia in children.

Pulmonary Tuberculosis. Artificial pneumothorax should be considered under the following circumstances: In cases of unilateral disease with cavitation, or which remain pyrexial after absolute rest in bed for two or three weeks: In similar cases which, although responding to absolute rest, show renewal of signs of activity on moving or on rising from bed: In cases of hæmoptysis which cannot be checked by other methods of treatment, and where the side from which the bleeding is occurring can be determined with a fair degree of accuracy: In unilateral acute pneumonic or broncho-pneumonic tuberculosis and in certain carefully

selected cases of bilateral disease in which the activity is mainly confined to one lung. Bilateral artificial pneumothorax is also of value in certain cases of bilateral pulmonary tuberculosis.

Bronchiectasis. The occurrence of a brilliant cure is especially likely in early cases before pleural adhesions have formed. This applies particularly to those cases resulting from the inhalation of some septic material, as may occur after operations upon the throat. Even in long-standing conditions of bronchiectasis, if unilateral, a determined attempt should be made to establish pulmonary collapse. The establishment of an artificial pneumothorax is also a preliminary stage for the operation of lobectomy if adhesions do not prevent it.

Interlobar Empyema and Localised Pulmonary Abscess. Artificial pneumothorax may be considered in those cases in which an interlobar empyema or abscess has ruptured into a bronchus, and the pus is not completely expectorated, the sputum remains offensive, and constitutional disturbance is present as indicated by pyrexia. Here, again, pleural adhesions may render the operation impossible or unsuccessful, as only a partial collapse is obtained. In such an instance an attempt may be made to cauterise the adhesions with the use of a thoracoscope.

The Method

The requirements are as follows :—

Pneumothorax Apparatus. There are several types in use. The Lillingston and Pearson apparatus is that which we usually employ. It has the advantage of simplicity, and can be used both for inserting air and also for removing it in cases of spontaneous pneumothorax.

The arrangement of the pneumothorax apparatus can be seen by a reference to the diagram, and a detailed description is not necessary (see Fig. 26). A and B are glass bottles, B being graduated in ml. from 0 to 1,100. C is a glass manometer. D is a four-way glass connection. E is a glass connection. F and G are glass filters containing sterile wool. H, I, J and K are glass tubes. L, M, N, O and P are rubber tubes. Q is the needle. 1, 2, 3 and 4 are clips on the rubber tubes.

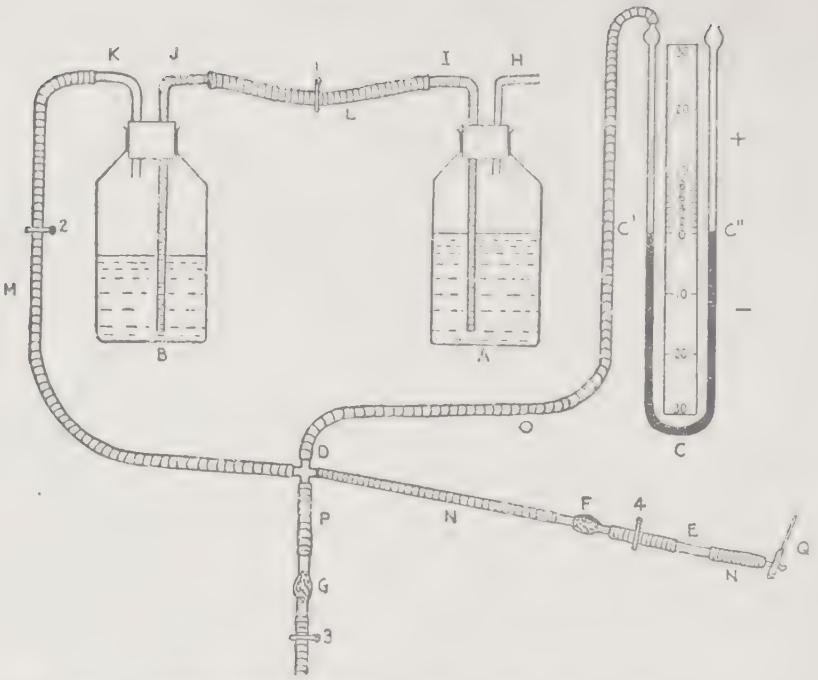


FIG. 26. Diagrammatic representation of Modified Lillingston and Pearson Pneumothorax Apparatus. The bottle B is graduated in ml. from 0 to 1,100.

Pneumothorax Needles. The needle used for the primary induction is often referred to as the No. 1 needle. The form devised by Clive Riviere is commonly employed. It consists of a blunt-ended cannula with a thin edge for penetrating the

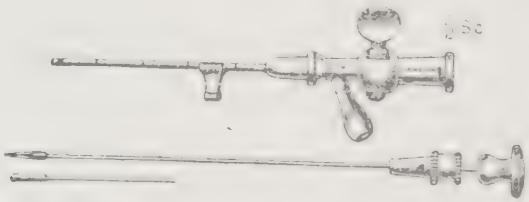


FIG. 27. Clive Riviere Needle (No. 1 Needle).

pleura, and a trocar for piercing the tissues of the chest wall. The needle used for refills is of a different pattern; it may be referred to as the No. 2 needle. The Saugman pattern is the one we use. This is shorter than the No. 1 needle and has a bevelled cutting edge like an ordinary hypodermic needle. It is provided

with a stilette for clearing purposes. The needles are illustrated in the text (see Figs. 27 and 28).

A 2 ml. syringe and hypodermic needle of sufficient length to reach through the chest wall to the pleura. (This distance is rarely more than 3 cm.)

Sterile 2% Novocain solution (procain. hydrochlor. B.P.).

Iodine, spirit lamp and sterile towels.

Some restorative, such as brandy, strychnine or adrenaline should be at hand.

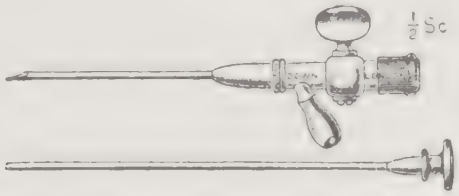


FIG. 28. Saugman Needle (No. 2 Needle), with Rubber Cap to act as a Stuffing-box.

Preparation of the Apparatus. The glass bottles, rubber tubes, glass tubes and glass filters are all sterilised by boiling. The needles are sterilised by placing them in spirit and subsequently flaming. The manometer is filled to the zero point with water coloured with a little red ink. This does not stain the glass and makes readings easy. The glass filters are filled with sterile cotton wool. The tubes are placed in position. Bottle A is filled with coloured solution of perchloride of mercury (1/1000), or with 1/100 carbolic acid. The fluid is passed over to bottle B, by blowing a little air into bottle A with a Higginson syringe attached to the tube H, which starts the siphon action. With the clips 1, 2 and 3 open the fluid in the bottles can now be run from one to the other by raising or lowering one of them. Half the fluid should be left in bottle B and half in A. The apparatus should be examined to see that there are no leaks before use. This can be done by closing clips 3 and 4 and opening clips 1 and 2. If, now, bottle A is raised, air will be driven from bottle B into the manometer and the fluid in limb C′ depressed. The level in the manometer should remain constant as long as the bottle A is kept in the same position. In the same way, if bottle A is lowered the fluid in limb C′ will rise. It can easily be ascertained that air can be driven through the pneumothorax needle by

opening clips 1, 2 and 4 and closing 3. If the end of the needle is now put under methylated spirit, having withdrawn the trocar and closed the side tap, air will be seen bubbling from the end of the needle on raising bottle A.

Preparation of the Patient. The patient is in bed. He should be given an aperient the night before the operation, and the induction should not be made immediately after a meal. Half an hour before the operation an injection of $\frac{1}{4}$ gr. morphin. sulph. should be given, in order to calm the patient and diminish any tendency to pleural shock. If the pneumothorax is to be induced on the right side of the chest the patient lies on his back turned towards his left side, with the right arm forward above the head, which is kept low, and a pillow is placed under the chest and left shoulder, so as to widen the intercostal spaces. The site of election for the first puncture is in the sixth space in the anterior axillary region. The skin over this area is painted with iodine, and sterile towels placed on the bed-clothes and against the chest.

The Operation. The Novocain solution is injected, about 2 ml. being used. The skin over the intercostal space is held taut with two fingers of the left hand. The needle is then inserted obliquely, just in front of the fingers, and a little Novocain injected intradermally to cause a bleb. After a short pause the needle is moved into a position at right angles to the chest wall, and gradually pushed through the intercostal space. Novocain is slowly injected as the needle moves downwards to the pleura. Care should be taken to inject the solution right down to the pleura, as this probably plays a part in the prevention of pleural shock which may cause a fatal result.

The patient must now be warned against coughing during the operation. If the desire to cough is irresistible, due warning must be given, otherwise the sudden increase in the pleural pressure when the patient coughs, with the pneumothorax needle in the pleural cavity, would drive the fluid out of the manometer. This can be prevented, if warning is given, by squeezing the tube N, leading from the needle to the manometer, or by closing the clip 4.

The rubber tube N leading to the manometer is attached to the side arm of the No. 1 needle. Clips 1, 2 and 3 are closed and clip 4 opened. With the trocar in position the needle is pushed

through the skin at the anæsthetised spot, and through the intercostal muscles, for about 1 cm. or less if the patient is very thin. It must be held firmly in the hand, with the top of the trocar in the palm, and not in the position of a pen between the fingers. The trocar is now withdrawn and the stopcock turned, so that the lumen of the needle is in communication with the tube leading to the manometer. The cannula is now pushed on through the remainder of the intercostal muscle down to the pleura.

When it is in contact with the pleura, but not through it, an oscillation will probably be observed in the manometer, not directly synchronous with respiration and of small extent. The cannula is now pushed through the pleura, which can often be felt to give way with a snap. Great care must here be taken not to penetrate the lung. When the cannula is in the pleural cavity the fluid in the limb C″ of the manometer falls below zero and oscillates with respiration. These oscillations correspond accurately with the respiratory movements, falling with inspiration in the limb C″ of the manometer and rising with expiration A reading is now taken, each centimetre of fall of fluid in the limb C″ corresponding with a change of pressure in the pleural cavity of 2 cm. of water. The oscillations should be at least 3 to 6 cm., and are often as much as 10 cm. Thus a reading of — 10, — 2 may be obtained, giving a mean negative intrathoracic pressure of — 6 cm. water. The respiratory fluctuations are greater when the patient takes a very deep breath, but the reading should be taken with ordinary moderately deep breathing. The needle may become blocked during its passage through the deeper parts of the chest wall, in which case it can be cleared with the stilette which is supplied with the No. 1 needle. The visceral layer of the pleura may also come in contact with the end of the cannula and cause obstruction. Letting in a small quantity of air by opening clips 1 and 2 is the best means of dealing with such a condition. When it is certain that the end of the cannula lies free in the pleural cavity, air may be admitted. This is accomplished by opening the clips 1 and 2, when the air is sucked from the bottle B into the chest, and fluid passes over from bottle A into bottle B to take its place. When about 100 ml. have thus passed in by suction, the bottle A can be raised slightly

and another 200 ml. of air admitted. The clips 1 and 2 are then closed, and the manometric readings taken for inspiration and expiration, with the patient breathing quietly and moderately deeply as before. They will still be negative, unless the air has been injected into a small loculated pleural space. These readings may be — 8, — 1. The mean pressure would in this case be — 4·5. The pressure changes, and the amount of air injected, would then be recorded as follows : — 6, 300 ml., — 4·5. It is to be noted that with this pneumothorax apparatus readings of intrapleural pressure cannot be taken while air is flowing in. The clips 1, 2 and 3 must be closed in order to obtain a reading. The needle is now withdrawn from the chest and a little iodine again applied to the site of the puncture. A collodion dressing is not usually required. If there is a tendency to cough, a large pad and a firm chest binder should be used in order to minimise extravasation of air. The patient is kept in bed at rest for the first month of pneumothorax treatment.

Refills. The same apparatus is required as for the initial pneumothorax operation, but a No. 2 needle (Saugman) is used. The patient is prepared as before, but it is not usually necessary to give a preliminary injection of morphine unless he is very nervous. It is wise, however, not to omit the anæsthetisation of the pleura with Novocain, as death may follow from pleural shock in refills as well as during the primary induction. A spot close to that used for the induction is chosen. The rubber tube leading to the manometer is attached to the side arm of the No. 2 needle with the stilette slightly withdrawn, so that it does not protrude beyond the point. The pneumothorax apparatus has already been prepared with the fluid in bottle B up to the 0 mark, the remainder being in bottle A. The clips 1, 2 and 3 are closed. The needle is now pushed straight through the chest wall at the anæsthetised spot into the pleural cavity. Directly it is felt to enter, the stilette is withdrawn and the stopcock closed, so that the lumen of the needle is in communication with the manometer. The fluid in the manometer will now oscillate. The pointed needle is used, as the air introduced at the primary induction (on the previous day) will prevent the lung being injured.

The reading is then taken. This will be found to be lower

than the final reading at the previous injection, as some of the air introduced has been absorbed. Clips 1 and 2 are now opened and air allowed to enter the pleural cavity. After about 200 ml. have passed in, the clips should be closed and a reading taken to make sure that the needle is in the pleural cavity and that the pressure is not rising too quickly. About 400 or 500 ml. will probably be enough for the first refill, in order to reduce the negative pressure in the pleural cavity by about 2 or 3 cm. of water. Thus the readings at the first refill may be — 5·5, 400 ml., — 2. If there are many adhesions, and the lung is not collapsing well, a smaller quantity of air should be required to reduce the negative pressure to a similar degree. The needle is then withdrawn.

The spacing of refills and the determination of the correct amount of air to be injected are matters of extreme importance, and are comparable with the administration of a vaccine. In neither case is it a matter of routine ; in each the personal factor of the patient is of paramount importance. Usually the second refill is given two days after the first, and the third three days after the second. The interval can then be gradually lengthened to a week, ten days, a fortnight, three weeks and a month. High intrapleural pressures should be avoided, as they cause mediastinal displacement, possibly tend to promote pleural effusion, and seem to have a direct relation to loss of weight on the part of the patient (Burrell and Garden (22)).

If there is a rise of temperature after the initial induction, the first refill should not be given the next day. The temperature should be allowed to subside first. A rise of temperature just before a refill is due probably indicates that the refills are being given at too long intervals, and that the lung is starting to re-expand (Burrell (23)). A rise of temperature after a refill is sometimes an indication that too much air has been injected. An X-ray should be taken at the end of the second week to determine the degree of pulmonary collapse, and the subsequent treatment should be subjected to X-ray control, in order to determine what degree of collapse is necessary to produce the optimum effect, as judged by temperature, pulse and body weight, and also so that the range of intrapleural pressure which produces such collapse can be ascertained. It is not usually

necessary to use a positive pressure to produce satisfactory collapse.

Some Difficulties and Complications. The manometer has been described as the heart of the pneumothorax apparatus. It is certainly of vital importance, not for the actual introduction of the air, but for its safe introduction.

The character of its oscillations affords the only reliable guide as to whether the end of the needle lies free in the intrapleural space. These typical movements have already been described.

Certain abnormal events may occur, in which case no air must be admitted into the chest :—

A negative pressure may be obtained on inserting the needle into the chest, but the fluid in the manometer may show this as a stationary negative pressure, without any respiratory excursions. This indicates that the needle has been in the pleural cavity, but is now either blocked or in the lung. The negative pressure may increase for a little with each inspiration, without any oscillations occurring if the needle is in the lung. The pressure may be slightly positive during expiration at the primary induction and negative during inspiration, indicating that the needle is in a bronchus or small cavity in the lung. The manometer may show a positive pressure which rises slowly or rapidly, indicating that the needle is in a blood vessel. Blood may then be seen passing through the glass connection E in the rubber tube attached to the needle. The pressure may be negative, but show irregular respiratory oscillations if the needle is in a pleural cavity much loculated by adhesions.

From such examples as these, it will be realised how important it is to be certain that the manometer indicates that the needle is in the pleural cavity before any air is admitted.

Complications. *Pleural shock* may occur, especially if the patient is very nervous and if the pleura is not anæsthetised.

Surgical emphysema may develop within a few hours of the injection of air, either as the result of coughing or of injury to the lung, or because the air has been injected extrapleurally between the pleura and the intrathoracic fascia. If a lung, which is bound down to the chest wall by adherent pleura, is punctured, surgical emphysema may develop without the introduction of any air.

Gas Embolus. This may occur from introduction of air into

a vessel, especially a pulmonary vein, in which the pressure is negative.

Effusion. This is especially liable to occur if adhesions are present and if high positive intrapleural pressures are used. About 50% of pneumothorax cases develop an effusion in the pleura during some time of their treatment. This may be: *Small*, giving rise to little constitutional disturbance, and often unrecognised except by X-rays. *Large*, causing constitutional disturbance with pyrexia. The fluid is serous, rich in cells and frequently contains tubercle bacilli. It indicates an active tuberculous pleurisy. *Infective*, often accompanied by rigors. This is pyogenic in origin.

In any case pleural adhesions are likely to occur both at the apex and the base, and it is improbable that a satisfactory collapse will be obtained afterwards, unless the fluid is repeatedly removed as soon as it forms and is replaced by air. The apical adhesions form because the fluid runs up to the top of the pleural space when the patient lies down.

The Treatment of Spontaneous Pneumothorax

In cases of spontaneous pneumothorax in which pressure symptoms are urgent, it has been the custom to allow the air to escape through a needle inserted into the pleural cavity.

The use of the Lillingston and Pearson apparatus enables the intrathoracic pressure to be determined before and after the air is removed, and also gives a reading of the volume of air that has been abstracted. It may therefore be employed with advantage.

For this purpose the bottle B is filled with fluid and the bottle A is almost empty. The chest wall and pleura are anæsthetised with Novocain, as for pneumothorax induction, and a No. 1 needle inserted into the pleural cavity. The intrathoracic pressure is then read, the clips 1, 2 and 3 being shut and 4 open. A reading such as $-8 + 10$ may be obtained, indicating a mean pressure of $+1$. The fluid in bottle B is now allowed to run over by siphonage into bottle A by opening the clips 1 and 2, and air from the chest replaces the fluid in the bottle B, the air in bottle A being expelled through tube H. Sufficient air should be removed to relieve the intrathoracic tension and produce a final pressure, which is

slightly negative. In the example quoted, after removal of 400 ml. of air, the readings were − 8 + 6, a mean of − 1. The needle is then removed from the chest.

If the pressure subsequently rises, as is often the case with a valve-opening in the lungs, more air can be removed in a similar manner on a subsequent occasion. If the pressure rises very rapidly continuous drainage can be employed. For this purpose the needle is left in the pleura, held by strapping to the chest wall. Alternatively the needle is passed through a metal collar attached to a flexible piece of metal. The position of the needle in the collar is fixed by a screw and the metal is strapped to the chest wall (Foster-Carter (24)). One of the tubes (O) from the four-way glass connection (see Fig. 26) allows the pressure to be recorded from time to time, by releasing a clip on it, whereas tube P from the connection passes down to end in a glass tube under disinfectant fluid in a bottle on the floor. The clip between the needle and this tube is kept open so that air can continuously escape under the fluid and thus the pressure in the pleura is prevented from rising excessively. This method is effective until the valve opening or bronchial fistula is sealed over. Subsequently, if the patient coughs, air is driven out from the pleural space and the fluid from the bottle rises higher in the glass tube, owing to the increased negative pressure in the pleura. This tends to re-open the communication between the lung and the pleura. Further, when this communication closes the increased negative pressure tends to cause the lung to expand rapidly and a previously satisfactory artificial pneumothorax may be obliterated. In order to

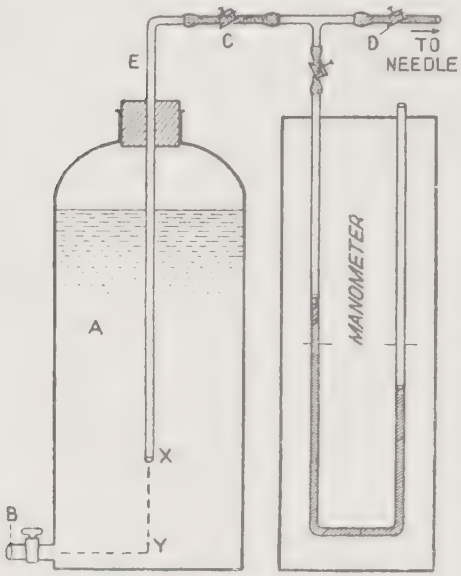


FIG. 29. The apparatus used in the treatment of spontaneous pneumothorax.
(Marriott and Foster-Carter, *Lancet.*)

prevent this, Marriott and Foster-Carter (25) have adapted "Mariotte's bottle" for use in the treatment of spontaneous pneumothorax (see Fig. 29). The bottle A should hold about 4 pints. The outlet B at the bottom of the bottle must be provided with a straight tap; if it is curved it may act as a syphon. The tube E, which passes through a rubber cork under the level of the water in the bottle, must slide easily through the cork so that its height can be readily altered. When the tap B is opened, air will be drawn in from tube E. If tube E communicates with a closed cavity such as the pleura, a negative pressure will develop within it, proportional to the height of its lower end X above the level of the outflow Y. Thus if the distance X—Y is 10 cm. a negative pressure of 10 cm. of water will develop in tube E when tap B is opened. The glass tube communicates by rubber tubing with a glass T tube, one arm of which communicates by rubber tubing with the pneumothorax needle strapped in the chest, and the other arm with a glass manometer. It can thus be used with a pneumothorax apparatus or with a separate manometer as shown in Fig. 29. The level of tube E should be so adjusted that a slightly negative pressure is produced in the pleura, sufficient to relieve the patient's distress but not so great as to cause re-expansion of the lung. In cases of spontaneous hydropneumothorax, the fluid can be simultaneously removed through an aspirating cannula inserted into one of the lower intercostal spaces.

It must be remembered that, after removal of gases from a pleural cavity, the whole of the pneumothorax apparatus will require sterilisation before it is used for another patient. This is because the gases from the pleural cavity have passed into the bottle B. The bottles, tubing, and filters should be dismantled and boiled. The filters are then refilled with sterile wool and fresh perchloride solution put into the bottles.

Gas Replacement of Pleural Effusions

On April 25th, 1882, Parker (26) demonstrated to the Fellows of the Royal Medical and Chirurgical Society, London, an apparatus for injecting filtered and carbolised air into the pleural cavity in order to facilitate aspiration in cases of empyema. He also referred to a case which had been successfully treated by this means. Judging from the subsequent discussion, the sugges-

tion was not very favourably received. The operation is now, however, a recognised and valuable method of treatment for certain cases of pleural effusion.

Gas replacement consists in the removal of fluid from the pleural cavity through one of the lower intercostal spaces towards the back, and, at the same time, the introduction of air to take its place through one of the intercostal spaces higher up and in front.

This treatment is of most value in cases of tuberculous pleural effusion, but is at times used for pneumococcal empyema, and for effusions due to malignant disease. In tuberculous pleural effusions it is of value for the following reasons :—

The lung is not allowed to ré-expand. Any pulmonary tuberculous focus is thereby kept at rest and adhesions are prevented from forming between the two pleural layers. These would probably render the subsequent establishment of an artificial pneumothorax impracticable.

When the mediastinum has been severely dislocated by a large effusion, the removal of the whole of the fluid may not be possible owing to symptoms of shock appearing as the heart passes back to its normal site. Gas replacement allows the fluid to be removed without a sudden readjustment of the mediastinum.

The pleural cavity can be almost completely emptied. This tends to lessen the probability of adhesions subsequently forming.

Gas replacement diminishes the tendency to the re-formation of pleural effusions, which so frequently takes place after simple aspiration. The method is therefore of value in the treatment of chronic recurrent pleural effusions.

In suspected cases of malignant disease of the lung, mediastinum or pleura, complicated by a pleural effusion, a gas replacement of the fluid is of value in allowing a clearer X-ray picture to be taken for diagnostic purposes. It probably does not relieve pain, if withdrawal of the fluid alone fails to do so, although it is sometimes used for this purpose. The fluid is best removed with a Potain aspirator.

Apparatus. Aspirator.

Pneumothorax apparatus with No. 1 and No. 2 needles.

Hypodermic syringe and needle.

Iodine, gauze and collodion.

Sterile 2% Novocain solution (procain. hydrochlor. B.P.).

Operation. The skin over one of the intercostal spaces is painted with iodine at the site judged to be most favourable for the removal of fluid, either in the posterior axillary line or in the mid-axilla. The patient, lying slightly on the sound side, is propped up with pillows. The skin, muscles and pleura are now anæsthetised with 2 ml. of the Novocain solution, as for pneumothorax induction. The pulse rate is counted. The No. 2 needle is now introduced, and a little fluid removed. A No. 1 pneumothorax needle, attached to the tube of the artificial pneumothorax apparatus, is now inserted into the pleural cavity in front, above the level of the effusion. The intrapleural pressure is then recorded. If this is high, above a mean of + 4 cm. water, fluid is run out of the chest to lower it before any air is introduced. Air is now passed in to take the place of the fluid removed by aspiration. The rate of the admission of the air is so regulated, by raising or lowering the bottle A, that the intrapleural pressure is kept constant.

If by any chance the pneumothorax needle should have been inserted into fluid, either free or pocketed in the pleura, the fluid will be observed passing through the glass connection (E) in the rubber tube (N) attached to the pneumothorax needle. It can be prevented from reaching the filter (F) by closing the clip 4 (see Fig. 26). Before allowing air to enter now it is advisable to run off some fluid through the aspirating needle. The pulse should be taken from time to time to see that it does not increase in rate unduly. If this occurs the operation should be stopped. When all the fluid has been run off, the intrapleural pressure should be left slightly lower than it was at the beginning. It will usually be found that less air has been introduced than fluid withdrawn even if the final pressure is equal to the original one. On removal of the aspirating needle, a gauze and collodion dressing should be applied.

Lipiodol in Diseases of the Lungs
(*Bronchography*)

It is not possible to visualise clearly the outline either of the bronchi and their ramifications or of pulmonary cavities by means of a direct X-ray examination. If, however, some substance

opaque to the rays is introduced into the bronchi, their configuration is clearly revealed.

Lipiodol or Neo-Hydriol (ol. iodisat. B.P. Add.) is a compound of iodine in poppyseed oil, which contains 40% iodine. It is a transparent oil of a light brown colour, which is opaque to the X-rays, and so heavy that it sinks in water. On prolonged exposure to air it darkens and should not then be used for injection. Sicard and Forestier (27) in 1922, injected Lipiodol into the trachea in man, using a cannula introduced into the glottis with the aid of a laryngoscope, or else passing it through a curved needle inserted through the crico-thyroid membrane.

A straight needle attached to a syringe may be used for the injection, and this method is now frequently employed, the Lipiodol being introduced either through the crico-thyroid membrane, or directly into the trachea below the cricoid cartilage. We usually employ the crico-thyroid route. Alternatively the Lipiodol may be injected orally between the vocal cords, using a curved syringe, after preliminary cocainisation. Some workers prefer to use a catheter passed through the nose into the larynx.

The Indications. X-ray photographs of the lungs after intratracheal injection of Lipiodol afford a graphic representation of the position of the trachea and main bronchi, of cavities in the lung connected with patent bronchi, of bronchial and pleural fistulæ, and of the relative permeability of the bronchial fields in different portions of the lungs.

Thus, it will show displacement of the trachea and bronchi due to pulmonary fibrosis or other causes, the presence of bronchiectasis in its various stages, and whether or not the lumen of the bronchi is obliterated by obstruction within or without, such as that caused by a foreign body, new growth or fibrosis. Lipiodol injections are of value in suspected cases of bronchiectasis, to determine whether there is a cavity present, and, if so, the extent of lung involved. They also show the condition of the apparently sound lung. This is of great importance in the treatment of bronchiectasis, especially in those cases in which it is impossible to establish an artificial pneumothorax on account of pleural adhesions, and in which a surgical operation, such as lobectomy, is contemplated. A lobectomy for

FIG. 30. Radiogram of lung after injection of Lipiodol, showing normal and dilated bronchi.

To face p 255.

bronchiectasis would generally be contra-indicated if the Lipiodol injection showed that the opposite lung was also the seat of bronchiectatic changes. In children, Lipiodol injections are chiefly of value in distinguishing between cavities due to bronchiectasis and those caused by tuberculosis. In definite cases of tuberculosis it is considered by some inadvisable to inject Lipiodol, for fear of spreading the disease.

The X-ray appearance of the lung in bronchiectasis after Lipiodol injection is shown in the accompanying plate (see Fig. 30).

Apparatus. Minim syringe with a fine needle (No. 7, hypodermic).

Minim syringe with a stouter needle (No. 2, hypodermic).

Lipiodol syringe. This must be strong, as considerable pressure is required to force the oil through the needle. We employ a 25 ml. syringe with three finger-rings, the needle being straight (No. 15 S.W.G., $\frac{3}{4}$ inch long), bevelled, with a bayonet mount which fits on a bayonet catch on the syringe.

Sterile 2% Novocain solution (procain. hydrochlor. B.P.).

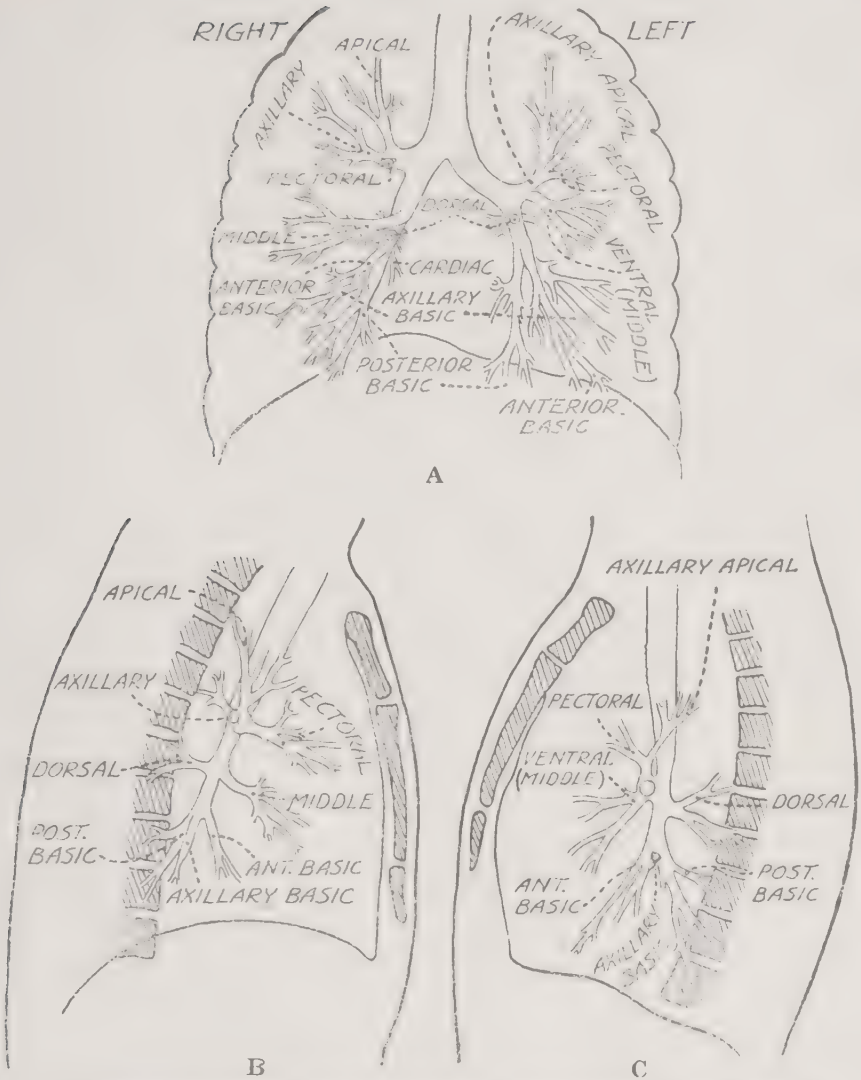
Sterile 5% cocaine solution.

Lipiodol warmed in a hot water bath to 99° F.

Iodine and wool.

The Operation. Preliminary tests should be made to determine whether the patient is sensitive to iodine or to cocaine. Ten grains of potassium iodide are given by mouth, t.i.d., for three days, to see if iodism results, as judged by lachrymation and nasal catarrh. Lipiodol or Neo-Hydriol rarely produce toxic effects in patients intolerant of iodides, unless they enter the stomach. In patients who are sensitive an erythematous rash may appear one to three days after the operation, or an urticarial rash about the tenth day. If the patient is sensitive it is wise not to proceed with the investigation as a fatal result may ensue. Preparations of oil containing bromine, which were formerly used in cases of iodine sensitivity, are no longer available. After a satisfactory iodide test, m. 2 of 5% cocaine solution are injected subcutaneously, idiosyncrasy to the drug being shown by faintness, dyspnœa, palpitations or cramps. If these tests are satisfactory the operation of bronchography may be performed. If the patient is nervous he should be given an injection of

morphin. sulph. gr. $\frac{1}{4}$ half an hour previously. Children under ten years of age usually require a general anæsthetic, such as ethyl chloride and ether. It should be of sufficient depth to abolish the cough reflex. The operation should be carried out in the X-ray room or in a room adjacent. The patient lies in a semi-recumbent position on an adjustable table, supported by three or four pillows beneath the shoulder blades, the head and neck being straight, the head extended as far as possible and resting on a sand bag. The skin of the neck is painted with iodine, the position of the crico-thyroid membrane identified, and the skin, subcutaneous tissues and crico-thyroid membrane anæsthetised by injecting $\frac{1}{2}$ to 1 ml. of 2% Novocain solution through the fine needle. Ten minims of 5% cocaine solution, warmed to body temperature are then injected rapidly into the larynx through the crico-thyroid membrane, using the stouter needle. A few bubbles of air should be sucked back into the syringe before the injection is made, to ensure that the needle is in the larynx. The needle is withdrawn immediately after the injection so that it is not broken when the patient coughs. The patient is now told to sit up and spit out the cocaine. He must not swallow any of it, and he should cough until he no longer feels a desire to do so. It is most important that he should not cough after the Lipiodol has been injected or the radiograms will be failures. The patient now resumes the semi-recumbent position, with head extended as before. The Lipiodol, which has been warmed to body temperature, is poured into the special syringe, and the needle introduced through the crico-thyroid membrane. The larynx is steadied during this procedure by the operator's free hand. The piston is withdrawn a little and a bubble of air is sucked back to verify that the needle is in the correct position in the larynx. This should be done subsequently before injecting the Lipiodol, after changing the patient's position. The patient is now turned slightly to the side which it is desired to fill, and he is instructed to breathe quietly through the mouth. Five to 8 ml. of Lipiodol are then injected, and about half a minute is allowed for them to run down to the bronchi of the lower and dorsal lobes. With the needle still *in situ* the patient is carefully sat up and inclined slightly forwards and to the same side, and a further 4 to 5 ml. of the iodised oil are injected. The patient remains in this position



A. Antero-posterior view showing the distribution of the bronchi.
B. Lateral view, showing the distribution of the bronchi on the right side.
C. Lateral view, showing the distribution of the bronchi on the left side.

(Nelson, *Brit. Med. Journ.*)

FIG. 31.

for another half-minute so that the middle lobe bronchi may be filled. He is then again placed in the semi-recumbent position, but he lies now completely on his side, and a further 4 to 5 ml. of iodised oil are introduced, and the needle is then withdrawn.

A few seconds are allowed for the oil to reach the orifice of the upper lobe bronchus. The patient's feet are now raised and the head lowered, in order that the upper lobe bronchi may be filled. The pectoral branches are best filled by turning the patient for a few seconds into the prone position. The bronchi on one side should now be completely filled and X-ray films are taken in the antero-posterior and lateral positions with the patient recumbent, and in the antero-posterior position with the patient erect. If a bronchogram is also required of the other side of the chest, the Lipiodol is introduced in exactly the same manner. It should be remembered that on the left side, as the upper lobe bronchus comes off rather more anteriorly, the patient should be inclined rather more to the prone position during its filling. Films are taken in the antero-posterior position, with the patient recumbent and erect. The one-stage technique described above is usually satisfactory, and avoids the necessity of two separate operations. The affected or more affected side should be filled first, and radiograms taken in the lateral and antero-posterior positions; the other side is then filled and antero-posterior radiograms only are taken. The anatomical distribution of the bronchial tree should be borne in mind; this was depicted by Nelson (28) (see Fig. 31).

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CHAPTER X

THE SEX HORMONES

The active principles of the ovary, testis and pituitary are now available for therapeutic purposes. In addition, various synthetic analogues of the gonadal hormones have been discovered. The physiological and therapeutic activities of these substances are discussed below, while fuller accounts of the isolation and chemical characterisation of the various hormones may be obtained by reference to the more detailed views mentioned in the bibliography.

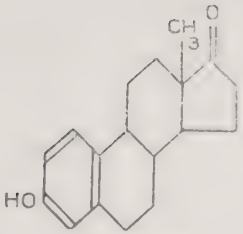
The Ovarian Principles

Natural Œstrogens. Substances capable of producing heat, or œstrus, in ovariectomised rats or mice, are called œstrogens.

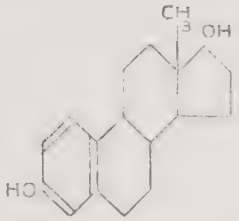
The most common naturally occurring substances having this property are œstradiol and œstrone. Œstradiol was first prepared chemically from œstrone, but was later isolated from follicular fluid by Doisy et al. (1), and it is now generally believed to be the œstrogen actually produced by the ovary. A number of other naturally occurring œstrogens have been isolated but are of more theoretical than therapeutic interest.

International units have been set up for both œstrone and œstradiol; one international unit of each substance being 0·1 microgram. Since, however, the two substances are pure chemical compounds, clinicians and research workers usually give dosages in terms of weight rather than units.

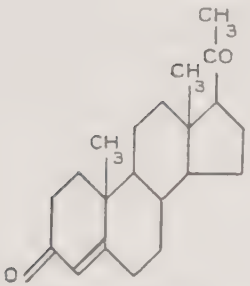
Œstrogenic Esters. Both œstrone and œstradiol are destroyed rapidly in the body (Zondek (2)), and, for this reason, if continuous therapy is desired, injections have to be made at frequent intervals. It has been found, however, that esterification of the two substances markedly delays their absorption so that the effect of a single injection may be greatly prolonged. The esters most commonly used are œstrone and œstradiol benzoates. The international unit of œstradiol benzoate is 0·1 microgram of the pure



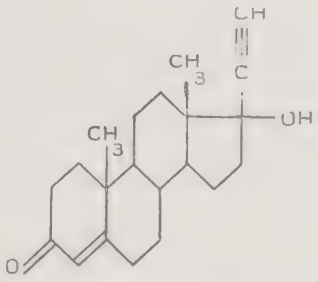
Œstrone



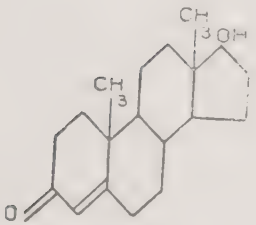
Œstradiol



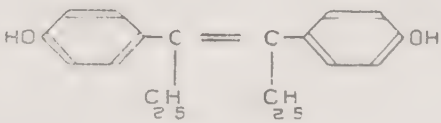
Progesterone



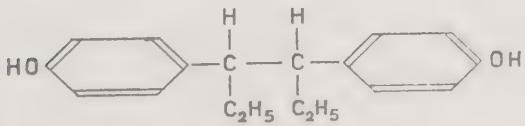
Pregneninolone



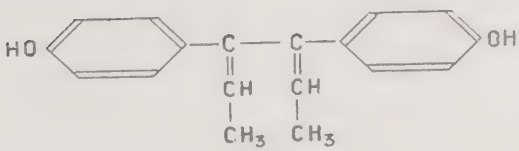
Testosterone



Diethyl-stilboestrol



Hexœstrol



Dienœstrol

compound, but, again, expression of dosage in such units is little used. Esterification may also serve to enhance the oral activity of the natural œstrogens. Ethinyl œstradiol has been shown by Inhoffen et al. (3) to have the same degree of activity by mouth as œstradiol has on subcutaneous injection.

Synthetic Œstrogens. Cook, Dodds, Hewitt and Lawson (4) showed in 1933 that it is possible to produce synthetic substances having œstrogenic properties, though only when very large doses are used. These substances all contained the phenanthrene nucleus and were thus chemically somewhat similar to the natural œstrogens. Later, Dodds and Lawson (5) showed that this complex ring system is not essential for physiological activity, and a large number of simpler synthetic œstrogens were obtained. These researches culminated in the discovery by Dodds, Robinson and their co-workers (6) of a substance, diethyl-stilbœstrol, with a relatively simple chemical structure and an œstrogenic activity greater than that of œstrone. Diethyl-stilbœstrol was found to be nearly as active by mouth as by injection, differing from the natural œstrogens in this respect; otherwise the new compound had all the physiological properties of the naturally occurring substances.

Stilbœstrol. In January, 1939, the first clinical reports of the use of stilbœstrol appeared (the diethyl prefix of the new compound was soon dropped). These tests were undertaken on behalf of the Therapeutic Trials Committee of the Medical Research Council and were published by Bishop, Boycott and Zuckerman (7), and by Winterton and MacGregor (8). A large number of confirmatory reports have been published in France, Germany, Scandinavia and America showing that stilbœstrol is capable of replacing the natural œstrogens in the treatment of amenorrhœa, dysmenorrhœa, genital hypoplasia, senile vaginitis, and menopausal disorders. Its cheapness and oral efficacy have led to its widespread use. Stilbœstrol dipropionate has also been used in therapeutics with success (Bishop et al. (9)).

Toxicity. The first clinical reports from America by Shorr et al. (10) on stilbœstrol claimed that the substance was very toxic. Among the symptoms mentioned were severe liver damage, jaundice, exfoliative dermatitis, and acute nervous symptoms. Such reactions have not been noted in this country or on the

Continent, and later American reports failed to confirm them. Stilbœstrol has now been released for sale in the U.S.A. Morrell (11) in 1941 reviewed over 257 articles on the use of stilbœstrol.

Hexœstrol. This is a hydrogenated form of stilbœstrol with equivalent activity, but, it is claimed, with less liability to cause the nausea and vomiting which ensue in some cases while the correct dosage of the synthetic œstrogen is being assessed (Bishop et al. (9)).

Dienœstrol. This compound is also closely related to stilbœstrol. Barnes (12) has reported that it is more effective than stilbœstrol in suppressing lactation.

Biological Actions. The chief biological actions of œstrogens are as follows: (*a*) Production of œstrus in ovariectomised animals. This includes enlargement of the uterus with endometrial proliferation without progestational changes, and vaginal cornification. (*b*) In large doses œstrogens cause an inhibition of hypophyseal activity so that the gonads may be decreased in weight, growth cease, and all other symptoms of hypophysectomy may be produced apart from the adrenal atrophy; the adrenals are actually hypertrophied after large doses of œstrogen. (*c*) In male animals large quantities of œstrogen may cause enlargement of the prostate with urinary retention and overflow. (*d*) In monkeys, bleeding may occur from the uterus following discontinuance of œstrogen administration. This bleeding, called œstrogen withdrawal bleeding, is also found in women.

Clinical Applications. *The Menopause.* The most common clinical use of the œstrogens is in the control of the disturbing symptoms so often present at the menopause. The vasomotor and psychic upsets that occur are attributed to the decline in œstrogen secretion by the ovary, and it is found that if œstrogens are administered to the patient in decreasing amounts over a period of about one year (although there are individual variations in the time), the symptoms are suppressed during the course of the therapy and do not recur when it is finally discontinued. The dosage necessary varies from 0·1 to 1·0 mg. of œstradiol benzoate injected weekly, or 1·0 to 5·0 mg. of stilbœstrol given daily by mouth. These doses are the higher limit and can generally be reduced with benefit. This is particularly so with the synthetic

œstrogen which tends to produce nausea and vomiting when given in too high a dose. Other conditions associated with the menopause, such as kraurosis vulvæ, and senile and leucoplakic vaginitis, are also benefited by œstrogen therapy. Details of these treatments will be found in the various papers by Bishop (7, 9 and 13).

Indication that the œstrogens are having physiological and not merely psychological effect is given by the appearance of the vaginal smear. This is examined by the method of Papanicolaou and Shorr (14), in which a specimen of the vaginal fluid is taken from the upper part of the vagina by means of a glass pipette with a rubber bulb attached to produce suction. The fluid is spread as a thick film on a glass slide and fixed in equal parts of ether and 98% alcohol before being stained with hæmatoxylin, eosin, and water blue. Papanicolaou has shown that the menopausal smear contains few cells with little evidence of keratinisation of the epithelium; following œstrogen administration, the smear changes to the post-menopausal condition in which there are many epithelial cells, a large proportion of them being keratinised.

Amenorrhœa. The use of œstrogens in the treatment of amenorrhœa has, on the whole, given disappointing results. This is not entirely unexpected in view of the great complexity of the factors governing the menstrual cycle. The old classification of amenorrhœa into primary and secondary types has proved to be inadequate. The present opinion is that œstrogens are only of value in the treatment of infantilism in the female; their administration has caused in many cases the appearance of secondary sexual characteristics and enlargement of the uterus. If the condition is primarily one of infantilism menstruation may commence. A course of 5 mg. of œstradiol benzoate spread over five weeks is usually adequate. Similar results have followed the oral administration of 14 mg. of stilbœstrol (Bishop et al. (7)). In dealing with so-called primary amenorrhœa, stilbœstrol should be given in fairly high dosage (2 to 5 mg. three times daily) for two to three weeks followed by an interval of a week to a fortnight when œstrogen withdrawal bleeding may take place. Treatment should be continued for several months, even after bleeding has occurred, so that the natural cycle may have time to become

established. In any treatment of amenorrhœa it is advisable to give a small dose of œstrogen periodically to avoid retrogressive changes in the uterus.

Suppression of Lactation. The action of œstrogens in suppressing the lactation of parturient women is most probably due to a suppression of hypophyseal activity. A single dose of 5 to 10 mg. of stilbœstrol has been found to be effective if given within twenty-four hours of the birth. If lactation is already established large doses may be necessary (Wenner and Jöel (15)). Barnes (12) has shown that dienœstrol may be more potent than stilbœstrol in this respect.

Treatment of Malignant Disease. A new and entirely unforeseen use of the synthetic œstrogens has come to light recently. Castration for the relief of carcinoma of the prostate was originally advocated by John Hunter who had observed that when the testes were removed the prostate gland would decrease in size. Interest in this form of treatment has been revived from time to time but has died down again owing to the high mortality rate and the undesirability of such an operation from the patient's point of view.

The possibility of bringing relief of carcinoma of the prostate gland by a modification of the endocrine system was first mooted by Huggins and his colleagues (16). In the four years from 1939 to 1943, Huggins treated sixty-nine patients with advanced carcinoma of the prostate by castration combined with the administration of œstrogens. He found that for this purpose the synthetic œstrogens were just as suitable as the naturally-occurring substances. Œstrogens were administered to patients who did not obtain prolonged remission of their symptoms after castration and also in those cases where the serum level of acid phosphatase, though considerably reduced, did not drop to normal. In the majority of cases so treated, symptomatic relief was obtained in a short time. Pain due to pressure on nerves was relieved as was also the frequency due to urinary obstruction, and there was usually a great improvement in the general condition of the patient, as shown by an increase in appetite, gain in weight and a rise in the hæmoglobin level. In addition to this, the primary tumour and the secondaries were often found to have decreased in size.

Nesbit and Cummings (17) also report a series of 75 cases

treated by orchidectomy. They also administered stilbœstrol to a few of these cases. Sixty-five of the patients obtained some benefit from the operation, but in many of the cases the benefit was only temporary. In 2 of the cases symptoms recurring after orchidectomy were relieved by stilbœstrol.

Many other workers have since treated carcinoma of the prostate with œstrogen therapy alone, and in a large proportion of the cases benefit has been obtained. Herrold (18) reported 12 cases treated with stilbœstrol alone and found that there was either stabilisation or regression of the carcinomas and an improvement in the general condition.

Kahle, Ogden and Getzoff (19) reported a series of 7 cases which had been treated for over two years with stilbœstrol. In all these cases prompt relief of pain and urinary symptoms were obtained and there was a regression of the malignant tissues, both primary and metastatic.

Kearns (20) treated 37 cases with stilbœstrol and/or ethinyl œstradiol. In 14 of the cases no operation was done. Five of the patients had previously been treated elsewhere by castration. Out of all 37 cases, 23 showed definite improvement, 11 showed partial improvement and 3 showed no improvement.

Marquardt and Flaherty (21) treated a total of 33 cases. Ten of these had no other treatment but stilbœstrol and 5 of these showed definite clinical improvement. Two patients were castrated after showing no benefit from stilbœstrol, but the operation also brought no benefit. Nineteen patients were treated by castration alone, and of these 11 were definitely benefited.

Later reports of cases followed up for more than two years suggest the possibility that in a large number of cases the improvement obtained by androgen control, whether by the administration of œstrogens or by orchidectomy, is only temporary. Nesbit and Cummings (17) pointed out that some of their cases must be regarded as "delayed failures," since the improvement was not always maintained even when œstrogens were given after castration. Herger and Sauer (22) report a series of 130 cases. They found that although 71 of the patients were still alive at the time of reporting, only 17 were without evidence of advancing disease. They also suggest that a considerable proportion of the

patients deriving benefit from the treatment at first will eventually be classed as delayed failures. Hooks (23) reports 27 cases, only 10 of whom were still alive at the end of two and a half years. For this reason it has been suggested by Bumpus et al. (24) that endocrine therapy should be held in reserve as long as possible and then resorted to by stages in order to prolong the period of relief as much as possible. It is too early yet to say with certainty whether the treatment is going to be of temporary benefit only in all cases, or whether there will be a certain proportion of cases that can be kept permanently symptom-free. Whatever the outcome may be, there is no doubt that in the cases which do benefit, even temporarily, the expectation of life is considerably prolonged and many patients who have been bedridden and in acute pain and discomfort have been enabled to resume their normal activities.

It is not possible to say yet exactly what is the mode of action of œstrogens in the treatment of carcinoma of the prostate. The changes in the serum level of acid phosphatase indicate that in some way the metabolism of the malignant cells is interfered with. This enzyme was found to be present in large quantities in the prostate gland of adult men by Kutscher and Wolbergs (25) and its presence was found by the Gutmans and their colleagues (26) to be dependent on the activity of androgens. Both the Gutmans (27) and Barringer and Woodard (28) found that the serum level of acid phosphatase is abnormally increased in some men with metastatic carcinoma of the prostate. Huggins et al. (29) showed that the presence of this enzyme in the prostate gland is really a secondary sexual characteristic of a chemical nature, and that the serum level can be reduced either by orchidectomy or the administration of œstrogens. Huggins also pointed out that in many cases a malignant prostatic tumour was due to hypertrophy of adult epithelial cells which are acted on by androgens. He therefore deduced that if the activity of androgens could be inhibited, either by removal of the testes or by the administration of œstrogens, then the growth of these cells would be interfered with and the tumour and possibly the secondaries would regress. One of the possible explanations for the failure of œstrogen therapy in some cases is that these tumours may be due to the malignant development of other cells on which

androgens have no controlling influence. A further explanation is the existence of an extragonadal source of androgens, possibly in the adrenal cortex.

It is still in doubt whether the action of synthetic œstrogens in prostatic carcinoma is entirely due to the inhibition of androgens or whether they exert some definite controlling influence on the malignant cells themselves. Schenken, Burns and Kahle (30) who did biopsies before and after treatment of 6 of the 7 cases reported by Kahle, Ogden and Getzoff (19) found that stilbœstrol had produced definite and characteristic changes in the cells and that these changes were different from the tissue changes caused by irradiation.

Until the mode of action of synthetic œstrogens in carcinoma of the prostate gland is known, it is not possible to say what is the likelihood of success in treating carcinomas in other parts of the body. A certain amount of success has been obtained in the treatment of carcinoma of the breast. A much smaller proportion of cases have benefited, but of a total of 168 cases reported by various workers in the Proceedings of the Royal Society of Medicine in October, 1944 (31), 41 were reported as having improved. In 6 cases the improvement was described as " spectacular." Again, the question arises whether the improvement in these cases is due to a change in the endocrine balance or whether it is due to the specific action of the drug on a certain type of cell.

A committee has recently been set up by the Royal Society of Medicine and the British Empire Cancer Campaign, the object of which is to investigate as large a number of cases as possible of carcinoma treated with synthetic œstrogens and to follow them up over a long period. The Report of this Committee cannot appear for a number of years, but it is hoped that it will yield much valuable information on the type of case most likely to be benefited and the reasons why such benefit is to be expected.

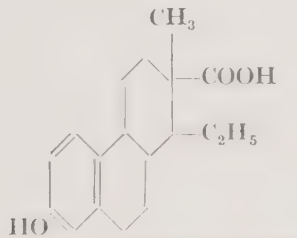
Certain side-effects have been reported from the administration of œstrogens for the relief of cancer. These include swelling, tenderness or soreness of the breasts, nausea, vomiting and occasionally soreness of the testes. As a rule, these effects are not serious enough to require cessation of therapy and they are generally far outweighed by the benefits obtained. Moore,

Wattenberg and Rose (32) made histological examinations of the breasts of patients treated with stilbœstrol and found definite changes in the appearance of the cells. These changes were not malignant and were not regarded as a contraindication to the use of œstrogens.

Dosage of synthetic œstrogens can be based either on symptomatic relief or on the serum level of acid phosphatase. In the cases which are benefited, 1 to 5 mg. per day is usually sufficient to bring about relief.

No serious worker in this field would suggest that synthetic œstrogens are a cure for carcinoma of the prostate or for any other form of cancer. Although in many cases a regression of the tumour and secondaries is obtained, and a definite change may be seen microscopically in the malignant cells, the disease begins to progress again if treatment is interrupted, so that the underlying cause of the malignant process, whatever that may be, is not eradicated. Nevertheless, œstrogen therapy does offer a means of treating with a simple oral remedy a condition which would either be untreatable or would necessitate a drastic operation.

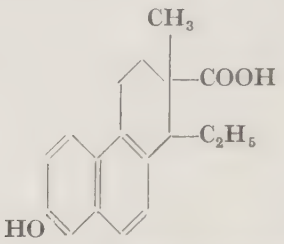
Doisynolic Acid. The activity of the early phenanthrene compounds investigated by Cook, Dodds, Hewitt and Lawson (4) was of a very low order, and it is therefore with very great interest that one turns to the recent development in synthetic œstrogens containing the phenanthrene ring system. In 1933 Doisy et al. (33) described a phenolic monobasic acid obtained from œstradiol by fusion with KOH, which was claimed to have several times the œstrogenic potency of œstrone. Miescher (34) has named this "doisynolic acid" and proved it to be 7-hydroxy-2-methyl-1-ethyl-1 : 2 : 3 : 4 : 9 : 10 : 11 : 12-octahydro-phenanthrene-2-carboxylic acid :



Doisynolic acid

It produces œstrus in rats in doses of 0·7 to 1·0γ

The corresponding naphthol derivative, bisdehydro doisynolic acid, has been prepared by total synthesis, and two racemates have been isolated, one of which is said to be active in doses of 0·1 to 0·15γ subcutaneously and 0·1 to 0·2γ orally :



Bisdehydro doisynolic acid

It is as yet too early to give any judgment on the clinical use of these compounds. They appear to be of the same order of activity in rats as the stilbœstrol series, and it will be interesting to see whether they have any advantages over the synthetic œstrogens of simpler structure now in use. It will be remembered that most workers hold the view that any side-effects of orally active œstrogens are associated with the œstrogenic potency and cannot be dissociated from it.

Possible Carcinogenic Effects. The natural œstrogens bear some chemical relation to the carcinogenic hydrocarbons. So far, however, no carcinogenic activity has been demonstrated by the methods commonly employed to test carcinogenicity in the hydrocarbon series. Lacassagne (35) has shown that the incidence of mammary carcinoma is increased, in strains of mice having a spontaneous incidence of such tumours, by the injection of large quantities of œstrone for long periods. It must be stressed that the doses were large, continued for a large proportion of the life span of the mice, and had no effect on mice not subject to spontaneous incidence of mammary carcinoma. This action of the œstrogens is quite different from that of the carcinogenic hydrocarbons since they do not act locally. There is no evidence that œstrogen therapy in human cases has any carcinogenic action nor is there any likelihood of such action, but the facts do suggest that œstrogen should not be administered unrestrictedly as has been sometimes the case, but the dosage should be carefully adjusted so that the desired effect is produced with a minimum dose.

This should be particularly the case in patients with a family history of mammary carcinoma.

Methods of Administration. In 1937 Deanesly and Parkes (36) showed that tablets or crystals of the naturally occurring œstrogens could be implanted subcutaneously in experimental animals, and that, by this method, small amounts of œstrogens were absorbed continuously over very long periods. This method of administration has been used clinically by a number of physicians, such as Salmon et al. (37). The subsequent discovery of orally effective œstrogens such as stilbœstrol and ethinyl œstradiol has removed the chief advantage of such a method of administration which, though it saves the patient a large number of injections, rendered the accurate control of doses somewhat difficult. Œstrogens may also be given by inunction and are sometimes given in the form of vaginal suppositories. These latter usually contain 0·1 mg. of crystalline œstrone made up in a base buffered to *p*H 4·5 to 5·0 suited to the reaction of the vagina. They are recommended for the treatment of the various forms of vaginitis.

Corpus Luteum Hormone

The secretion of the corpus luteum is responsible for producing a thickening of the uterine mucosa and increase in its glandular complexity. These changes normally occur in the second half of the menstrual cycle, the so-called progestational phase. This action of the corpus luteum hormone can only be produced when the uterus has been previously sensitised and stimulated to the requisite stage by œstrogen. These progestational changes are essential for the implantation of the fertilised ovum. The active principle responsible was first demonstrated by Corner and Allen (38) and later obtained in a crystalline state. It belongs to the group of steroid substances and is called progesterone. It is inactive when given by mouth but a derivative synthesised in 1938 by Hohlweg and Inhoffen (39) is orally effective. Chemically, this substance is ethinyl testosterone, and was called by its discoverers pregneninolone ; the British Pharmacopœia has given it the official name of ethisterone.

Biological Action. The function of progesterone in the body is concerned with the preparation of the uterus to receive the fertilised ovum and to maintain pregnancy when the ovum has

become implanted. These actions have been worked out in some detail in experimental animals and it is found that progesterone has little if any effect on the uterus unless the latter has already been sensitised by preliminary treatment with œstrogens. It should also be noted that œstrogens will inhibit this action of progesterone so that the uterine changes of the normal menstrual cycle depend upon the successive action of œstrogen and progesterone, and progestational changes will not occur unless progesterone is present and œstrogen absent. Experiments in animals have shown that the progesterone is also necessary for the maintenance of the early stages of pregnancy.

Clinical Applications. *Menstrual Cycle.* From the above considerations it is obviously possible to reproduce the menstrual cycle in amenorrhœic women, and Kaufmann (40) successfully produced normal menstruation in bilaterally ovariectomised women by giving five œstrogen injections spaced over fifteen days, followed three days later by five daily injections of corpus luteum hormone. Certain clinicians have reported that progesterone injections are beneficial in cases of dysmenorrhœa, metrorrhagia and menorrhagia. It is difficult from the data at present available to make a final decision as to the value of this therapy. There is little doubt, however, that in some cases good results may be obtained, the usual dosage being 1·0 to 5·0 mg. progesterone daily.

Threatened and Habitual Abortion. The chief use of progesterone in human therapy has been in the treatment of cases of habitual or threatened abortion. Statistical analyses by Malpas (41) and MacGregor and Stewart (42) have shown that women who have aborted spontaneously three times are almost certain to abort again, and results obtained in patients who have aborted less often than this are statistically of little significance. Another difficulty in assessing the results of treatment is that these cases are usually treated with rest and other medicaments, chiefly vitamin E. It is generally agreed at the present time, however, that progesterone therapy is of use in such cases, although the evidence for it is by no means overwhelming. Five to 10 mg. daily are given when the symptoms of threatened abortion are present, or during the first three months of pregnancy in habitual abortion. Claims have been made that pregnanediol

glucuronide excreted in the urine is an index of the likelihood of abortion taking place. This substance is the normal excretion product of progesterone from the body and is assayed in the urine by a fairly simple method devised by Venning (43). This author and others have shown that many cases of spontaneous abortion are associated with a lowered excretion of pregnanediol glucuronide compared with that in normal pregnancy, and also that there may be a dramatic drop in the excretion during signs of threatened abortion. Unfortunately these excretion figures are by no means constant and cases have been reported by Hain (44) and Cope (45) of women with extremely low pregnanediol outputs who have had entirely normal pregnancies, and of drops in the excretion unaccompanied by any overt signs of threatened abortion. Thus, while pregnanediol excretion is used as an index of the function of the placenta (which, it is generally agreed, supplies most of the progesterone produced in the body during the later months of pregnancy), it is not an absolutely reliable prognostic aid.

The Testicular Hormone

Substances causing development of the secondary sex characters in immature or castrated male animals are known as androgens. The two most common androgens are androsterone, which is present in the urine of normal males, and the more active testosterone which is isolated from bulls' testes. The latter compound is most used in therapeutics and is prepared synthetically. As with œstrogens, esters of testosterone are more slowly absorbed than the parent compound, and testosterone propionate is the most common androgen used in clinical practice. Latterly it has been found that methyl testosterone is active when given by mouth, and, since the effective dose by this method is of the same order as that of testosterone propionate given intramuscularly, its use is now recommended.

Biological Activity. The chief action of androgens in experimental animals is to promote the growth of the external genital organs, seminal vesicles, and other accessory sexual organs in the castrated or immature animal. The growth of the comb in the capon thus produced by inunction of androgens is used for their biological assay. Androgens in large doses, like œstrogens, will

depress the pituitary function and it is presumably this action which is responsible for the inhibition of lactation which they produce. Androgens are also said to antagonise the effect of œstrogens on the prostate.

Clinical Applications. *Eunuchs and Eunuchoids.* The chief use of androgens in medicine is in the treatment of castrated and eunuchoid individuals. The treatment is in no sense curative and acts purely as replacement therapy. The dosage varies from case to case, but is usually about 20 mg. of testosterone propionate given daily by intramuscular injection. By this treatment the genitalia, apart from the testes, are stimulated and eventually the size of the organs becomes quite normal. Hair growth on the face and body, which is practically non-existent in the castrated person, starts within a few weeks. The range and pitch of the voice are also altered and approach those of a mature man. As a side effect during androgen treatment, there is often a retention of nitrogen, and body weight and muscular development may be increased. The testes, however, do not assume normal function and when treatment is stopped the changes which have been produced regress, though maturation of the voice may persist.

A matter of debate is the age at which androgens should first be administered to children with evidence of sexual immaturity. The actual age at which puberty occurs shows considerable variation and some of these children, if left untreated, develop quite naturally. On the other hand too early treatment, especially if the dosage given is high, may result in premature closure of the epiphyses, resulting in stunting of growth. Since no form of androgen therapy has ever been shown to stimulate spermatogenesis in man, the only permanent changes which may be avoided are those in the skeleton; pre-pubertal eunuchs, if untreated, develop extremely long limb bones with late closure of the epiphyses.

Sterility. At the present time there is no evidence that sterility associated with aspermatogenesis can be cured by androgen therapy. Some claims have been made that sterility associated with a low sperm count may be benefited by administration of testosterone, but other authors have claimed that the sperm count may be reduced, presumably by hypophyseal depression.

The Prostate Gland. Experimental observation that œstrone

may cause enlargement of the prostate in mice was made by Lacassagne (46) and was soon extended to higher animals. In primates enlargement of the prostate may result in urinary retention and overflow. It has also been shown that this action of œstrone may be inhibited by testosterone or the prostate of the animal pre-treated with œstrone may be reduced in size. These experimental findings led some clinicians to administer testosterone in human cases of benign hypertrophy of the prostate. The results have been conflicting ; it should be pointed out that the prostatic enlargement occurring in elderly men is not histologically identical with that occurring in œstrone-treated animals and that many instances of spontaneous improvement occur.

Use in Women. Testosterone has been used in the treatment of metrorrhagia owing to its known action of preventing progestational changes in the endometrium. It is also claimed that testosterone is more effective than œstrogen in suppressing lactation once the latter has become established. In general, however, it is unwise to recommend androgens to women in any case where œstrogens or other female sex hormone may be effective. Androgen therapy in women may occasionally give rise to undesirable effects such as the growth of hair on the face, deepening of the voice and enlargement of the clitoris. Such masculinising effects may regress only very slowly after cessation of treatment.

Gonadotrophins

Substances which cause development of the ovaries in immature animals or in those which have had their pituitary glands removed are called gonadotrophins (or gonadotropins since either term is correct according to international agreement). These substances are derived either from the pituitary or from the placenta. Many investigators are of the opinion that there are two separate gonadotrophic fractions, one of which causes follicle stimulation in the ovary or stimulation of spermatogenesis in the testis, while the other causes ovulation and luteinisation of mature follicles, and interstitial-cell stimulation in the ovary and testis. These two fractions are present in varying proportions in gonadotrophic extracts derived from different sources. There are some experi-

mental findings which, however, do not support the claim that these are divisible. Li et al. (47) and Chow (48) have claimed the isolation of chemically pure follicle-stimulating gonadotrophin and luteinising gonadotrophin. Preparations which are available at present consist of relatively crude extracts prepared from animal hypophyses, the urine of pregnant women, or serum of pregnant mares. The pituitary extracts vary considerably in the relative proportions of follicle-stimulating and luteinising activity present in them; the gonadotrophin in pregnant mares' serum (usually called serum or equine gonadotrophin) is mainly follicle-stimulating; that from the urine of pregnant women (called chorionic gonadotrophin) mainly luteinising. International standards for the latter two gonadotrophins have been established so that dosage can now be described in terms of international units.

Clinical Applications. *Undescended Testes.* Chorionic gonadotrophin was first used in the treatment of cryptorchidism by Shapiro (49), and Engle (50) was able to show that this gonadotrophin caused descent of the testes in immature monkeys. In the years that followed, however, although many cases have been reported, they were by no means uniformly successful. The optimal minimum age for this therapy to start is nine to ten years, for, if chorionic gonadotrophin is given earlier, precocious puberty may be produced.

Sterility in Women. In spite of earlier encouraging claims as to the value of serum gonadotrophin in the treatment of sterility due to lack of ovulation, critical examination of the literature shows that success is attained with this preparation only in certain selected cases. Experimental evidence favours the view that ovulation or correct regulation of ovarian function can only be attained when the luteinising factor, such as chorionic gonadotrophin from pregnancy urine, is given in addition to the follicle-stimulating factor chiefly present in serum gonadotrophin; also that these two factors should be given successively rather than together and that their relative dosages will have to be judged individually for each case. It appears unlikely that preparations containing mixtures of various gonadotrophins will have much success.

Menstrual Disorders. There have been multitudinous reports

of the effects of gonadotrophic extracts as well as of gonadal hormones in the regulation of disorders of the menstrual cycle. The subject is an extremely complex one and the results obtained have been diverse. Each case must be assessed on its individual merits, and an attempt made to gauge whether the pituitary or the gonads are finally responsible. Certainly at the present time no general rules can be laid down whilst the subject is yet in the experimental stage.

The Aschheim-Zondek Pregnancy Test

This test depends upon the fact that the urine of pregnant women contains a substance which induces the formation of hæmorrhagic follicles in the ovary of the sexually immature female mouse. Pregnancy may be diagnosed as early as the first week after the first missed period.

Procedure. *The Animals.* Infantile female mice three to four weeks old (weighing 6 to 10 g.) are used. It is important that the age limitation should be adhered to, for the reason that the ovarian cycle in the mouse commences at about the sixth week of life. Five animals are used in each test.

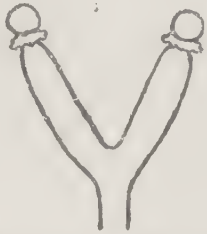
The Urine. The urine should be fresh, and, if possible, a morning specimen. If more than a day is likely to elapse between the passing of the urine and its arrival in the laboratory, it is recommended that a small amount of disinfectant be added (3 drops of lysol or tricresol per 100 ml. of urine).

The Injections. In the original description of the test, Aschheim and Zondek (51) recommended that each of the five mice should receive a different dose; thus the first would receive six doses of 0·5 ml., the second six doses of 0·4 ml., and so on. In our experience this practice has little to recommend it, for it is frequently found that the mouse receiving the largest dose gives a weaker response than others receiving smaller doses. For this reason we give all five mice six doses of 0·35 ml. The injections are spread over forty-eight hours as follows: first day, morning and evening; second day, morning, middle day and evening; third day, morning.

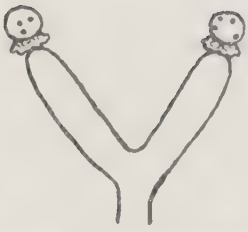
Results. (*a*) *The Ovary.* The animals are killed on the morning of the fifth day and their genital apparatus examined by the naked eye. Three types of reaction are observed in the

ovary. These are called by Aschheim and Zondek anterior pituitary reaction (Hypophysen Vorderlappen Reaktion) I., II., III. H.V.R. I. consists in the formation of large ripe follicles in the ovary. H.V.R. II. consists in the formation of hæmorrhagic follicles (Blutpunkte). H.V.R. III. consists in the formation of corpora lutea and/or of corpora lutea atretica. Reactions II. and III. separately or together constitute a positive result, whereas reaction I. alone does not. In practice it is usual to find all three

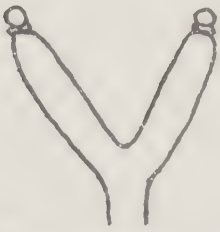
Normal control.



Reaction I.: Enlarged follicles with secondary uterine enlargement. Aschheim-Zondek test negative.



Reaction II.: Blutpunkte in ovary. Aschheim-Zondek test positive.



Effect of Œstrin: No action on ovaries.

FIG. 32.

reactions together, though occasionally a decision has to be made on the basis of H.V.R. II. alone. Where H.V.R. III. is present the diagnosis is easy, often, in fact, obvious. Where H.V.R. II. alone is obtained it is sometimes necessary to make use of a hand lens to decide whether or not a corpus luteum is present.

The diagrams illustrate the conditions which may be found (see Fig. 32).

(*b*) *The Uterus.* In the presence of reaction I. it is usual to find the uterus much enlarged, and sometimes distended with fluid. This, however, is not always found. It occasionally happens that the ovary is filled with corpora lutea with some "Blutpunkte," while the uterus closely resembles that of the untreated animal.

(*c*) *The Vagina.* The vaginæ of animals showing a marked reaction I. will be found to open. Vaginal smears are of the type characteristic of œstrus.

Difficulties of the Test. (1) *Toxicity of the Urine.* If it is found that the specimen of urine kills the mice before the completion of the test it can be detoxicated in the following manner, as described by Zondek (51). Take 30 to 40 ml. of morning urine and after making acid to litmus with acetic acid, filter. Shake with 90 to 100 ml. of ether (for anæsthesia) for five minutes. Separate off the ether in a separating funnel and allow the urine to stand in a wide-mouthed open vessel for an hour to get rid of most of the ether. If the ether is driven off in a water-bath the temperature of the urine must not be allowed to rise above 40° C. The urine when so treated will usually be well tolerated by the mice. In some cases we have found that centrifuging the urine at a high speed has rendered the urine harmless. Since the ether extraction of the urine removes the œstrone, the uterine and vaginal reactions will be considerably less, and attention must be concentrated on the ovary.

(2) *Time Consumed in Making the Test.* Zondek (51) describes a method by which the time required for the test is cut down from five to three and a half days. It should be noted that if the test is negative by the short method a repetition must be made by the original technique.

Errors of the Test. The error of the Aschheim-Zondek test amounts to 1 to 2%. Actually, the error in negative results is slightly higher than in positive. For this reason we make a practice of repeating the test in all cases in which negative results are obtained. Reaction I. is obtained apart from pregnancy in the following conditions : (*a*) after the menopause (25% of cases) ; (*b*) after castration, operative or X-ray ; (*c*) in cases of carcinoma, especially genital carcinoma.

A few cases of carcinoma in the male have been found to give

reaction I., and in two cases of carcinoma in the testes Zondek obtained reactions II. and III.

The Friedman Test (52)

As in the case of the Aschheim-Zondek reaction, this test depends on the formation of corpora hæmorrhagica through injections of prolan. Rabbits at least twelve to fourteen weeks old are employed, and these animals should be segregrated for a period of three weeks. In view, however, of the facility with which rabbits apparently spontaneously enter a period of pseudo-gestation it is essential to examine the ovaries by laparotomy before carrying out a test.

Procedure of Test. Ten ml. of filtered urine are injected intravenously into the ear vein. Twenty-four hours later a further 10 ml. of urine are injected. Twenty-four hours later the animal's abdomen is opened and the presence of corpora hæmorrhagica indicates a positive reaction. It is wise to test the sensitivity of the rabbit to pregnancy urine if a negative response is obtained, and the uterus is infantile and the ovaries small.

As in the case of the Aschheim-Zondek test, the Friedman test is positive in other conditions than pregnancy ; these include hydatidiform mole, chorion epithelioma, and certain teratomata of the testicle. In hydatidiform mole and chorion epithelioma, prolan is present in the urine in much greater amounts than in pregnancy, and the former conditions may be diagnosed by diluting the urine before injecting it into the rabbit, as follows : The urine is diluted 1/100 and 1/200. Ten ml. of the diluted specimens are injected on two successive days as in the original procedure. The presence of corpora hæmorrhagica forty-eight hours later indicates a positive test for hydatidiform mole and chorion epithelioma. An ordinary pregnancy urine diluted 1/100 would not produce corpora hæmorrhagica.

Advantages of the Friedman Test. (1) A result may be obtained in forty-eight hours. (2) Rabbits are easier to obtain than immature female mice. (3) The larger ovary of the rabbit allows the reaction to be noted with much greater ease than in the case of the mouse.

Gonadotropic Hormones in Cases of Testicular Tumours

The urinary excretion of gonadotropic hormones in cases of testicular tumours has been studied by Ferguson (53). He made a quantitative determination of the amount of gonadotropic hormones in the urine, and on the basis of these estimations he divided malignant testicular tumours into the following five groups in decreasing hormone concentrations: (1) Chorion epithelioma. (2) Embryonal adenocarcinoma. (3) Embryonal adenocarcinoma with lymphoid stroma. (4) Seminoma. (5) Teratoma with adult features.

Since Ferguson's investigations, other workers have stressed the importance of differentiating the types of gonadotropic hormone excreted in the urine, rather than merely quantitatively analysing the total hormones in the urine. Among others, Gerber (54), and Hamburger, Bang and Nielsen (55), have carried out work on the nature of the hormones excreted. These authors distinguished between the gonadotropic hormone found in and produced by the pituitary, and that present in the urine of pregnant women and produced by chorionic tissue. The first, or so-called *hypophyseal hormone*, produces, when injected in adequate amounts into immature female mice, a pronounced stimulation of the follicles and an increase in weight of the ovary. Normally this hormone is present in such small amounts in the blood and urine as to give no reaction with mice, but in certain conditions such as senility, castration, the menopause, and in some types of testicular tumours, there is an increased output and the typical changes are produced in mice, as described above. The second, or *chorionic hormone*, when injected into immature female mice produces the Aschheim-Zondek reaction with corpora lutea and blood points.

Hamburger has classified testicular tumours, according to the hormonal excretion, into four groups.

The group of pseudo-seminoma was made because, although the histological diagnosis was that of seminoma, clinically the cases did not conform to this type. Both cases were radio-resistant, and died within a year of the diagnosis.

No. of Cases.	Diagnosis.	Negative.	Traces.	Hypophyseal Hormone.	Chorionic Hormone.	Both Hormones.
42	Seminoma. . .	13	4	25	0	0
2	Pseudo-seminoma .	0	0	0	2	0
21	Mixed Epithelioma .	3	1	0	14	3
10	Polycystic Teratoid .	4	2	4	0	0

The value of determining the nature of the gonadotropic hormone excreted in the urine is thus clear both from a diagnostic and a prognostic point of view. It is generally recognised that the seminomatous type of tumour associated with an increased excretion of the hypophyseal hormone is radio-sensitive, so that it subsides completely when treated with X-rays, and the prognosis is relatively good. In the case of mixed epitheliomata, often associated with an output of the chorionic hormone, the prognosis is very bad and they are resistant to X-rays. After removal of a seminoma and successful radiation, the hypophyseal hormone still continues to be excreted in excessive amounts. A review of the literature on testicular tumours will be found in the paper by Gordon-Taylor and Till (56).

Urinary Hormones for Diagnostic Purposes

Although it may be assumed that androgenic and œstrogenic compounds are present in normal urine, it is not possible to identify these compounds with the active hormones. On the contrary, definite evidence accumulates that in neither of these cases is the true hormone excreted. Œstradiol which is regarded as the primary hormone is not detectable even in female urine. Instead the structurally related but physiologically less active compounds, œstrone and œstriol make their appearance.

A similar position is encountered in the case of the androgens. Testosterone, the primary androgenic hormone, has been isolated from bulls' testes, but even when directly injected as the propionate fails to gain access as such into the urinary tract. Three main excretory products are found in normal male and female urine, viz :

17 keto α steroids : (1) androsterone ; (2) 3 α hydroxyetiocholanone 17 (pseudo-androsterone)

17 keto β steroids : (3) dehydroisoandrosterone.

All three compounds possess androgenic activity in smaller degree than testosterone.

Urine extracts do not contain measurable amounts of progestational substances. Progesterone cannot be isolated from late pregnancy urine or blood as such, but it is a relatively easy matter to isolate the biologically inactive reduction product, pregnanediol. This is a case of inactivation and not of reduced activity.

Any attempt, therefore, to employ estimations of urinary hormones for diagnostic purposes must suffer from two major drawbacks. Firstly, the activity which urine possesses is not a true measure of hormonal metabolism but a coincidental phenomenon resulting from the lack of specificity amongst related steroids. Only in a crude sense can such figures mirror the transitions taking place in the endocrine glands. Secondly, such is the complexity of the steroids from urine that any figure obtained for activity must represent the sum of several related compounds differing amongst themselves in potency.

Extraction from Urine. The androgens and œstrogens of urine occur as conjugated water-soluble compounds from which they are liberated by hydrolysis. Hydrochloric acid brings about complete hydrolysis in one hour, but unfortunately results in the partial substitution of the hydroxyl group of dehydroisoandrosterone by chlorine to give 3-chloro-Δ^5-androsterone-17. This secondary reaction can be avoided by using sulphuric acid to bring about the hydrolysis, but even here there are indications of lowered results due, it is believed, to the production of hydroxyl-free unsaturated steroids.

Combined hydrolysis and extraction avoid the difficulties inherent in the above method. Carbon tetrachloride has been found to be a suitable solvent for removing the acid-sensitive steroids from the aqueous phase as they are formed. Various accounts suggest that hydrolysis and extraction are reasonably complete after half an hour with this solvent used in conjunction with hydrochloric acid.

The removal of the volatile solvent from the extract leaves the "steroids" as a dark brown gum. For further purification and separation this latter is taken up in ether and extracted with a saturated solution of sodium bicarbonate and then with 2N sodium hydroxide. The œstrogens, being phenolic, pass into the alkaline aqueous solutions from which they are subsequently recovered. The remaining ether solution, which contains the "androgenic steroids," can be further improved by extraction with a solution of 10% sodium hydrosulphite in normal sodium hydroxide.

The alkaline solution of the phenolic fraction is acidified with hydrochloric acid and cooled. The sodium bicarbonate washings are shaken twice with 50 ml. of ether, the ethereal layers being kept and the aqueous layer discarded. This ether is then used to extract the acidified solution of the phenolic fraction twice, using 50 ml. for each extraction, and a third extraction using fresh ether is then made. The ether extracts are combined, washed twice with 50 ml. of water, dried with anhydrous sodium sulphate and evaporated to dryness to give the final œstrogenic extract. In these extractions the purity of the ether is of great importance as the presence of peroxides in the ether has a destructive effect.

The processes used for the preparation of gonadotropic extracts from urine are based on adsorption on tannic acid or benzoic acid, the methods due to Thomsen and Pedersen-Bjergaard (57), and Katzman and Doisy (58) being used. It has been found that extracts prepared by the Katzman and Doisy process are very toxic when injected into rabbits, and the tannic acid process is now used in the laboratory of the Courtauld Institute, and is as follows : The sample of urine, which should be as fresh as possible, is adjusted to *p*H 4·6, using glacial acetic acid, and a 10% N/V solution of tannic acid, freshly prepared, is added with vigorous stirring, 25 ml. of solution per litre of urine being used, and the mixture allowed to stand in cold store overnight. The precipitate is then centrifuged off, washed four times with acetone, 50 ml. per litre of original urine being used for each washing, and dried in a vacuum desiccator. The resulting powder can then be dissolved in a borax potassium chloride buffer at *p*H 9·3, the solution being allowed to stand overnight in cold store, and any insoluble matter centrifuged off. The solution is then used for

injection. A further purification is possible by adding four volumes of alcohol to the solution with vigorous stirring, allowing to stand in cold store overnight, centrifuging off the precipitate and drying, after washing with alcohol and ether, in a vacuum desiccator. The powder can then be dissolved in water at neutral *p*H for injection.

In the preparation of these urine extracts the storing of the urine and its collection are of great importance. The urine should be immediately mixed with an efficient preservative, toluene and " Brilliant Green " being found very effective, and stored at 0° C. Thymol has been used for the preservation of urine, but this causes difficulties in the extraction, and hence its use should be avoided. When daily collections are being made, loss during defæcation should be guarded against. The importance of the storage of the urine can be shown by the reported loss of 36% of the androgenic activity of a urine that had been allowed to stand for one week at room temperature ; with toluene added as a preservative no loss of androgenic activity occurred.

Biological Assay of the Hormones

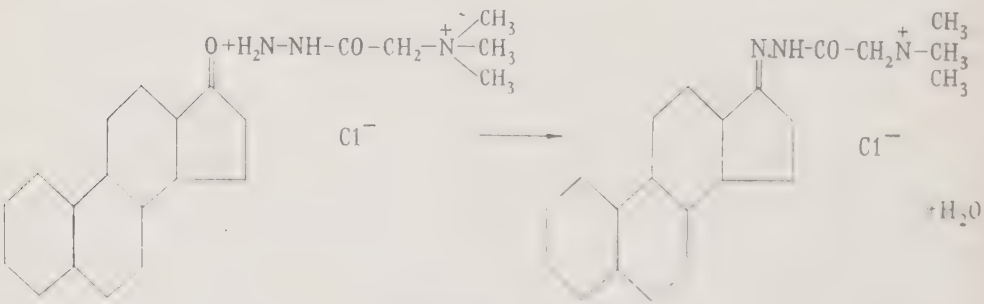
Androgenic. The normal method of assaying androgenic activity is based on the induction of growth in the comb of the capon, and the unit of activity is defined as the activity of 0·1 mg. of crystalline androsterone ; this weight is approximately the daily dose required to give an easily measurable response in the comb after five days. An increase in comb size of about 5 mm. in length and height, or 15% increase in area, is usually obtained from 1 international unit per day for five days. Detailed techniques have been described by Dodds, Allan, Greenwood and Gallimore (59), Schoeller and Gehrke (60), Freud et al. (61), Greenwood, Blyth and Callow (62), and Emmens (63). Other methods of measuring androgenic activity using mammals have been employed. The simplest criterion of androgenic activity in mammals is the increase in weight of the prostate and seminal vesicles of castrated rats, and this has been extensively used by Korenchevsky (64), Freud (65), Callow and Deanesly (66), and Tschopp (67). A disadvantage of this method lies in the fact that the several accessory glands respond differently to androgenic substances, and hence the activity of a single substance depends

on the organ used as a basis for comparison. Other methods depend on the survival of mobility of epididymol spermatozoa and the power to ejaculate.

Œstrogenic. The technique of Allen and Doisy (68) now consists in the injection of the ovariectomised test animal and the examination for the appearance of the characteristic cornified cells in the vaginal contents. In different laboratories the details of the technique vary, some workers using rats, others mice, and the number of injections given varies from one to eight. The extent of the confusion can be seen from the ratio of the mouse unit to the rat unit, values of 1 to 1/12 being found by various workers, and thus results between different laboratories cannot be compared unless the same detailed technique has been used. It is important to employ the same technique when assaying urine extracts for œstrogenic activity as that used in obtaining standard curves with the pure hormone. Detailed techniques have been described by Allen and Doisy (68), Allen et al. (69), Coward and Burn (70), Dodds et al. (71), and Curtis and Doisy (72).

Assay of Neutral 17 Keto-Steroids in Urine

Colorimetric. In the colorimetric assay of 17 keto-steroids proposed by Zimmermann (73), little claim to specificity can be justified. The colour given with meta dinitrobenzene in caustic soda solution is typical of all compounds containing the activated methylene grouping $-CH_2-CO-$; a grouping common to many compounds, steroid and otherwise. The specificity of the test lies in the extraction process and particularly in the separation of the Girard T complex (74) :



Girard T reaction

Complexes of this type are formed by all truly ketonic compounds. The presence of the polar group in such complexes endows the compounds with water-solubility and permits a sharp separation of ketonic and non-ketonic neutral steroid fractions.

If 17 keto-steroids alone were present in the neutral keto-steroid extracts, the estimation of androgens would be relatively straightforward. Unhappily this is far from being the case. The positions 3 and 20 are common sites in the steroid skeleton for the ketonic grouping; significantly the 3-position in many of the compounds isolated by Kendall (75) and Reichstein (76) from the adrenal cortex is occupied by a carbonyl group capable of carrying these compounds into the neutral ketonic steroid fraction.

Callow, Callow and Emmens (77) have shown that the absorption spectrum of the compounds containing the carbonyl group in the 17-position is characterised by a broad absorption band extending over the centre of the visible spectrum with a maximum in the green (5010 A°), whereas the 3 and 20 keto-steroids (under the conditions of colour development) give a low general absorption with a small peak in the yellow as well as in the green. According to Callow et al., the neutral keto-steroid fraction from most urine samples gives an absorption spectrum that closely resembles that of a typical 17 keto-steroid (androsterone) except that the extinction coefficient in the violet (Ev) is higher and of the same order as that in the green (Eg). When the ratio Ev/Eg exceeds 0·8, results should be interpreted with caution. Such urines are encountered and the results may be considered to furnish qualitative evidence of the presence of additional keto-steroids.

The androgenic fraction of neutral 17 keto-steroids has been shown to consist of mixed α and β steroids. Langstroth, Talbot and Fineman (78) have made use of the fact that β keto-steroids form an insoluble equimolecular complex with digitonin to separate further the neutral steroid fraction.

Polarographic. Compounds capable of being reversibly reduced at the surface of a dropping mercury cathode lend themselves well to polarographic estimation. Fieser et al. (79, 80) have shown that, although fully saturated keto-steroids are not reduced under these conditions, the Girard T complexes produced from them may be estimated polarographically in reasonably pure

solution. Without preliminary treatment $\alpha\beta$ unsaturated ketones (corticosterone, dehydrocorticosterone, desoxycorticosterone, etc.) are reduced and can be assayed by this method. Fieser et al. and Morris and Barnet (81), comparing the colorimetric and polarographic methods of estimating 17 keto-steroids, have found good agreement. When differences existed, the figure by polarograph was usually the smaller and possibly the more accurate. The difficulty of interfering 3 keto and 20 keto-steroids is also encountered in this method. Morris and Barnet recommend a preliminary oxidation with periodate and permanganate as a convenient solution of this difficulty.

Ketonic groups produced by Oppenhauer oxidation (aluminium isopropoxide) and periodate oxidation may act as a basis for the formation of Girard T complexes. This opens up further possibilities of the polarographic method. The following example serves to illustrate this application. The only important compound of the β steroid fraction obtained by digitonin precipitation of the neutral 17 keto-steroids from normal urine is dehydroisoandrosterone. Although a saturated ketone, this is converted by Oppenhauer oxidation to Δ^4 androstenedione, 3, 17, which as a Girard T complex gives a characteristic polarographic wave and lends itself to estimation.

Origin of Neutral 17 Keto-Steroids

Differences in normal values obtained by the various workers in this field are probably due to slight modifications in the chemical methods employed and the relatively small number of groups investigated. Normal figures given by Callow et al. (77) are :

Men	.	3·5–15 mg. 17 keto-steroid in 24 hours.
Women	.	1·7–12·6 mg. 17 keto-steroid in 24 hours.

Most workers accept lower values in juveniles and in old age. Ovariectomy in females has little or no effect on the amount excreted, but in Addison's disease (cortical insufficiency) the yield drops to zero. From this it may be inferred that in women androgens arise principally from the adrenal cortex. These findings are borne out by the corresponding estimations in men. Castrated males excrete somewhat less than normal, whilst in Addison's disease of the male, although a substantial fall in

excretion is found, the figure does not drop to zero. In the male it is probable that the adrenal cortex and the testes both contribute to the total androgenic fraction ; the consensus of opinion would seem to indicate that the former is the chief source. It is not certain whether this androgenic activity is coincidental to adrenal cortex metabolites or whether it is truly functional.

Compounds 1, 2 and 3 above have been isolated from the urine of both normal and gonadectomised males and females. Compounds 1 and 2 are isolated in lower yield from castrated males than from normals. These facts suggest that dehydroisoandrosterone is predominantly a product of the cortex. The intramuscular injection of testosterone propionate increases the excretion of 1 and 3, but not of dehydroisoandrosterone. The 17 keto α steroids, androsterone and 3-α-hydroxyetio-cholanone-17 probably represent the end products of testosterone metabolism.

Anterior Pituitary Lobe Hormones other than Gonadotropic

By a process of hypophysectomy on the one hand, and of grafting of anterior lobes and administration of extracts on the other, it is possible to demonstrate that the following hormones are secreted : (1) Growth. (2) Thyrotropic. (3) Adrenotropic. (4) Lactogenic. (5) Parathyrotropic.

In addition, it is possible to demonstrate a diabetogenic and ketogenic principle by other methods referred to later.

The Growth Hormone. It has been known for many years that if an animal is hypophysectomised before it has grown to full stature, growth will not take place. Evans and his collaborators (82) were able to show that the intraperitoneal injection of anterior lobe extract is capable of restoring the power of growth to such animals. Evans claimed that it is possible also to produce giant rats by this treatment. The growth hormone has successfully been extracted from the anterior lobe, and has been made available for experimental work. It is available for clinical trial in the form of antuitrin G.

The Thyrotropic Hormone. One of the most noticeable effects of hypophysectomy is a rapid atrophy of the thyroid. Administration of a suitable extract of the anterior lobe will cause a

rehabilitation of the thyroid gland. If excess is given then hyperplasia of the gland appears, and a condition very similar to Graves' disease will result. Exophthalmos has actually been described in animals treated with an excess of the thyrotropic hormone. It is interesting to note that even with absence of the hormone myxœdema does not arise, thus indicating that the thyroid gland has at least a certain autonomy of action. It is obviously tempting to suggest that the pathology of Graves' disease arises in the over-production of the thyrotropic hormone. At present, however, the most careful investigations of the blood and urine of persons suffering from this condition have failed to detect an excess of this substance. As will be noted later, on repeated injection the extract loses its power through the development of antihormones.

The Adrenotropic Hormone. A very similar state of affairs occurs in connection with the suprarenal. Hypophysectomy is followed by a decrease in the size of the cortex of the suprarenal glands; this being one of the characteristic signs of complete hypophysectomy. Administration of a suitable extract of the anterior lobe will cause enlargement and thickening of the cortex to its normal size. In mammals, signs of suprarenal insufficiency do not occur in hypophysectomised animals; but in certain birds such as the duck, hypophysectomised animals have to be treated with cortical extract in order to maintain life. Thus, as in the case of the thyroid, the suprarenal appears to possess a certain power of independent action, since symptoms of Addison's disease are never found in cases where the hypophysis has been removed.

The Lactogenic Hormone. This has been extracted by Riddle and his co-workers (83) from the anterior lobe of the pituitary, and its activity may be demonstrated by injection into pigeons. It is followed by hyperplasia of the crop glands and the secretion of milk. The hormone is capable of causing these changes in both sexes. It is suggested that this hormone is responsible for lactation at the end of pregnancy, and experimental work has shown that it is almost certainly responsible for conferring the mother instinct on animals.

The Parathyrotropic Hormone. Histological changes have been described in the parathyroid glands following hypophysectomy, and it is stated that they may be prevented by

administration of extracts of the anterior lobe of the pituitary. This has suggested the presence of a parathyrotropic factor.

The Diabetogenic and Ketogenic Hormone. In the year 1930, Houssay (84) made the quite remarkable observation that if a diabetic, depancreatised dog is hypophysectomised, the diabetes disappears and, in fact, the animal tends to suffer from hypoglycæmia. This observation has been confirmed by all the workers in this field, and such an animal is always referred to as a " Houssay preparation." If the injection of an extract of the anterior lobe of the pituitary is made into a Houssay dog its death from glycosuria and ketonuria will result. This led Houssay to postulate the presence of a diabetogenic and ketogenic hormone in the anterior lobe. It is difficult to be certain at the present time whether the two hormones are separate, but there is no doubt as to the experimental facts. Subsequently Young (85) succeeded in producing permanent diabetes in the dog by the injection of an extract of the anterior lobe of the pituitary, and this diabetes is of considerable interest in that it is associated with ketonuria. An examination of the pancreas showed a more or less complete absence of islets. There can be no doubt that the development of this work will be of the utmost importance to the understanding of the ætiology of diabetes.

It must be emphasised that these anterior pituitary principles have not been isolated in any degree of purity, but it is known that they all possess the general properties of proteins. They have not yet been made available in sufficient quantities for clinical trial. This whole subject is still in a state of flux. A review of the situation will be found in the paper by Houssay (86).

Anti-hormones. Some very interesting observations have been made by Collip (87) and his co-workers in Montreal. They have demonstrated that it is possible to produce in animals immunity to the action of some of the principles contained in the anterior lobe of the pituitary already referred to; for example, the thyrotropic hormone. It was found that whilst the first series of injections produced a definite rise in the basal metabolic rate, repeated injections failed to produce any effect. Collip has shown that the serum of such an animal, when injected into another, will confer upon it immunity to the thyrotropic hormone. This has led him to postulate the presence of an anti-thyrotropic

hormone. By injecting larger animals with thyrotropic hormone over a prolonged period it has been possible to accumulate large quantities of blood serum containing the anti-thyrotropic substance. Collip has found it possible to remove a great deal of the serum protein, and thus to obtain the potency in a highly concentrated form. The clinical trial of anti-thyrotropic hormone has up to the present time proved disappointing.

Posterior Lobe of the Pituitary

A connection between the posterior lobe of the pituitary and the stomach has been demonstrated by Dodds and Noble (88). It was shown that if an extract of the posterior lobe of the pituitary gland is made and injected into rabbits, then in twelve hours there is an intensive hæmorrhagic lesion of the acid-bearing area of the stomach. The animal usually recovers from this, but if several injections at intervals have been given, definite chronic lesions of the stomach may be produced. This factor is also contained in the Pharmacopœial extract, but it must be borne in mind that doses out of all proportion to the clinical ones must be administered. The actual significance of these observations is by no means clear at the present time, but they form a very interesting experimental demonstration that a relationship exists between the base of the brain, namely, the pituitary, and the stomach. It will be recalled that Cushing and others have described acute gastric lesions following operations on the base of the brain and the pituitary gland. It has also been reported by Dodds and his co-workers (89) that a severe anæmia of a pernicious type occurs in rabbits injected with large quantities of posterior lobe pituitary extract. It has been shown that this anæmia is not due to water retention or intravascular hæmolysis, but appears to be the result of an increased destruction of red cells.

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CHAPTER XI

THIOURACIL

In 1941 the MacKenzies and McCollum (1), while engaged in a purely scientific enquiry, chanced upon a discovery of considerable clinical importance. They were investigating the possibility that sulphaguanidine "when fed to rats on a purified diet containing synthetic B. vitamins, would prevent the synthesis of additional essential nutrients by the intestinal flora." They observed that after periods varying from six to sixteen weeks a hypertrophy and hyperæmia of the thyroid gland with loss of colloid was invariably produced. It was later found that this effect was not abolished by adding iodide to the diet, but could be neutralised by adequate doses of thyroxine.

Kennedy and Purves (2), in the same year, showed that rape seed and allyl-thiourea were powerful goitrogenic substances, and in 1943 the MacKenzies (3) found that thiourea and thiourea derivatives, especially 2-thiouracil, produced thyroid hyperplasia with inhibition of hormone production. Astwood (4) used thiourea and thiouracil in the treatment of patients suffering from thyrotoxicosis, and showed that the daily administration of 1 to 2 g. of thiourea, or of 0·2 to 1 g. of thiouracil, resulted in the relief of the patient's symptoms, and a return to normal of the serum cholesterol and basal metabolic rate, usually after a latent period of two to three weeks. These results were rapidly confirmed by Williams and Bissell (5) and by Himsworth (6). Both thiourea and thiouracil have a similar action in cases of thyrotoxicosis, but, because of the action of side effects, such as nausea, vomiting, and an unpleasant smell in the breath, which may accompany the use of thiourea, thiouracil, methyl-thiouracil or propyl-thiouracil is the drug which is now exclusively employed.

Mode of Action. Thiouracil produces its effect by inhibiting the synthesis of thyroxine, the colloid in the thyroid of the treated patient being devoid of iodine. It probably prevents the utilisation of iodine by the thyroid, as is indicated by the experiments

of Rawson et al. (7) with radio-active (tracer) inorganic iodine. The thyroid becomes hyperplastic, friable and vascular, and these changes are thought to result from excessive stimulation of the thyrotropic hormone formed in the anterior pituitary, as they do not occur in hypophysectomised animals treated with thiouracil. Further, the normal human thyroid is very little affected by thiouracil, thus Astwood (8) gave 400 to 600 mg. daily for several months to healthy individuals before producing any fall in the basal metabolic rate.

Clinical Applications. All types of thyrotoxicosis respond to thiouracil treatment, and, as has been described by Himsworth (9), Nussey (10), Reveno (11) and others, equally good results have been obtained in cases of primary and secondary Graves' disease, although the response may be slower with a toxic adenoma. Grainger, Gregson and Pemberton (12), on the other hand, found that primary cases responded much more favourably than do secondary ones, in fact, the longer the duration of the goitre, the less favourable the response to thiouracil. All observers agree that patients who have recently received iodine show a delayed response when treated with thiouracil. Thiouracil is equally efficacious in the treatment of thyrotoxicosis resulting from recurrence after thyroidectomy. King and Rosellini (13) successfully treated a case of acute thyroiditis, administering 200 mg. of thiouracil three times daily. The patient was free from symptoms in a week, and was cured after a further two weeks' treatment, taking 200 mg. daily. Thiouracil has also been used by Raab (14) in angina pectoris, and he found that 4 out of 10 cases were entirely free from symptoms during the treatment. Thiouracil is considered by some to be more efficacious than iodine in the pre-operative treatment of thyrotoxicosis, for by its use the surgeon can operate on a non-toxic goitre. Bartels (15) reports that it is especially of advantage in very severe cases of Graves' disease, especially those of long standing, which either do not tolerate, or fail to respond to, iodine therapy. Further, by giving Lugol's solution after the B.M.R. has been reduced to normal by thiouracil, the operation may be rendered easier, the gland being less vascular, less hyperplastic and less friable. Neither pregnancy nor diabetes are contra-indications to the use of thiouracil. In pregnancy it is wiser not to continue the thiouracil during the last month, but

to substitute it then by iodine, for in one case treated with thiouracil by Eaton (17) up to the confinement, the child at birth had an enlarged thyroid. Diabetes mellitus associated with thyrotoxicosis may be improved by thiouracil treatment, less insulin being subsequently required, but this is not so in every case, for Rose and McConnell (18) treated a thirteen-year-old girl with thiouracil, the result being that there was no increase in carbohydrate tolerance, and no diminution in insulin requirement.

Contra-indications. Thiouracil should be used with great caution if the goitre is causing sufficient pressure symptoms to warrant surgical intervention, as a rapid enlargement of the gland might result in a catastrophe. Thiouracil is contra-indicated if the patient has to go abroad after the stabilising treatment is completed, where adequate supervision with blood counts and basal metabolic readings are not available.

Stabilising Treatment. Some authorities do not keep their patients in bed during the initial period of treatment, allowing them to continue at work, providing adequate laboratory controls are carried out. We prefer the patient to be in hospital during the stabilising treatment, and in bed for the first three or four weeks. Before the thiouracil treatment is begun the following observations should be made: The weight of the patient, the pulse rate, the blood pressure, the circumference of the neck, the basal metabolic rate, the total white cell count and percentage of polymorphonuclear cells, and the blood cholesterol. The drug is given in tablet form by mouth, 200 mg. t.i.d. The white count is repeated twice a week during the early stages of treatment; the other observations, apart from the daily temperature and pulse record, being carried out weekly. Dried yeast tablets (D.C.L.) two t.i.d., may be given during the treatment, with the idea of preventing the development of agranulocytosis, but they are of doubtful value. The first effects of the thiouracil treatment are usually seen within one to two weeks, the lag being due to the time taken to use up the stores of thyroid hormone and prevent the formation of more. In some cases no improvement is noted for four to twelve weeks, especially if iodine has been recently given. As soon as the B.M.R. is falling satisfactorily, which is usually in about four weeks, the thiouracil dosage is reduced to 200 mg. twice daily, and later to 100 mg. three times or twice a

day. When the B.M.R. has fallen to between + 5 and — 10 (Aub du Bois) a maintenance dose of between 100 to 300 mg. daily should be given. The progress chart of one of our patients, a woman aged forty-six years with a small hyperplastic goitre is given below :—

THIOURACIL CASE SHEET

Date 1945	B.M.R. (Aub du Bois)	Blood Cholesterol mg./100 ml.	Leucocytes per cmm.	Polymorphs	Pulse per min.	Blood Pressure mm. Hg.	Wt. lbs.	Thiouracil mg.
Oct. 25	+44	158	7,800	68%	102	140/70	83	200 t.i.d.
Nov. 1	+32	192	6,300	numerous	100	128/68	87	200 t.i.d.
Nov. 15	+20	190	8,700	,,	78	132/72	96	200 t.i.d.
Nov. 21	+19	218	9,200	,,	84	110/80	99	200 t.i.d.
Nov. 29	+14	246	6,300	,,	88	112/60	103	200 t.i.d.
Dec. 6	+ 7	236	8,800	,,	84	120/65	108	100 b.i.d.
Dec. 13	+ 9	276	5,600	,,	76	118/66	111	100 b.i.d.
Dec. 20	0	250	10,000	,,	76	125/62	117	100 b.i.d.

A low white cell count before the commencement of thiouracil does not appear to be a contra-indication to its use. Thus in one of our cases the white cell count was 3,700 per cmm. with 55% polymorphonuclears before treatment, and during treatment the count rose to 5,100 per cmm. with 60% polymorphonuclears. This point has also been noted by Nussey (10). Auricular fibrillation may require simultaneous administration of digitalis, although some cases are restored to normal rhythm by the use of thiouracil alone, as recorded by McGavack et al. (16) in 12 out of 18 cases. If there is congestive failure it is wise to administer digitalis.

Overdosage of thiouracil is shown by marked increase in the size of the thyroid gland, or by symptoms of hypothyroidism such as a bloated appearance, a feeling of heaviness, lassitude and depression.

Maintenance Treatment. It is quite probable that the dosage of thiouracil will have to be frequently adjusted during the early stages of the maintenance treatment. The B.M.R. and leucocyte count should be determined every month until the condition is firmly stabilised, subsequently three-monthly readings should suffice. In some cases the thiouracil has been discontinued after a few months, and the patient has remained in good health for

periods up to eight months, as recorded by Eaton (17). In others relapse has rapidly followed lowering or omitting the dose of thiouracil. It is too soon yet to say whether thiouracil can lead to a permanent cure, but the general impression is to the contrary.

Toxic Reactions. These have been met with in from 10 to 20% of cases treated, but with the general use of smaller doses than were originally prescribed, they appear to be less frequent. The most dangerous complication is agranulocytosis, and unfortunately it may appear without any warning and rapidly prove fatal, despite regular control of the dosage of thiouracil by B.M.R. and white cell readings. In a survey of 1,091 cases treated at eleven clinics in America and at University College Hospital, London, Moore (19) reported that 19 patients developed agranulocytosis, of which 5 proved fatal. It is therefore probable that the mortality from agranulocytosis is about $\frac{1}{2}$% or less, and its importance must not be over-exaggerated. Wosika and Braun (20) reported a fatal case in a woman aged fifty years who had taken a total amount of 29·4 G. of thiouracil during eleven weeks, in average-sized doses. The response of the thyrotoxicosis to the thiouracil had been good. The symptoms of the agranulocytosis consisted of fever, sore throat with redness and a dirty white exudate, and restlessness. The patient died in coma in six days, the white cells falling to 500 per c.mm. with a complete absence of neutrophils. The usually accepted forms of treatment were of no avail; pentnucleotide, yellow bone marrow, ascorbic acid, thiamine hydrochloride, whole blood, liver, folic acid and penicillin. Cargill and Lesses (21) had a fatal case just a year after the initiation of treatment, which had been most carefully controlled throughout. The development of agranulocytosis appears to be a matter of idiosyncrasy, unpredictable and probably unpreventable, but fortunately rare. Moore (19) found that there is a period of maximal danger of agranulocytosis between the fourth and eighth weeks of treatment, so that at this stage particular vigilance is necessary, and it is also at this period that there is the greatest likelihood of leucopenia, by which is meant a white cell count below 3,000 per c.cm. without evidence of infection. Penicillin is worth a trial in any case of leucopenia, with the idea of preventing the secondary infection which so often proves fatal. Thus Rothendler and Vorhaus (22) successfully treated a case by

injections of 20,000 units of penicillin every three hours for four days, together with daily injections of 2 ml. of crude liver, and a whole blood transfusion of 500 ml.

Other toxic reactions make a formidable list, but fortunately are not often seen. They include drug fever, usually about the eighth day, morbilliform or urticarial rashes, allergic arthritis, nausea, vomiting, abdominal pains, diarrhœa, headache, enlargement of lymph glands or of the submaxillary glands, œdema of the legs, jaundice of an obstructive type, increase of exophthalmos, thrombocytopenic purpura, acute hæmolytic anæmia, heart-block, mental stupor, confusion and delusional insanity.

It has been suggested that methyl-thiouracil is less liable to produce toxic reactions than is thiouracil, but this is doubtful. Propyl-thiouracil is also considered to be less toxic. The initial dose is smaller, 50 mg. three times daily, until the B.M.R. falls, when the dose is reduced.

Results of Treatment. A good result is obtained in over 80% of cases of thyrotoxicosis treated with thiouracil. The failures are few in number, perhaps 4 or 5%, due either to lack of response to the drug or to intolerance. In the remainder of cases there is only a partial response.

Conclusions. Thiouracil is not a cure for thyrotoxicosis, for it does not affect the underlying cause of the over-activity of the thyroid gland. Striking as are its results it is not in every case to be preferred to surgery. In the majority of instances, however, thiouracil appears to be the treatment of choice. There is little difference between the risk of medical or surgical treatment. The operative mortality rate, in the best hands, is about ½ to 1%, the chance of death from thiouracil being possibly a little less. There are, however, other drawbacks associated with thyroidectomy; it is not infrequently followed by myxœdema and substitution therapy is then required. Other complications, such as tetany and recurrent laryngeal nerve palsy, are not unknown, and even the development of exophthalmic ophthalmoplegia.

By the successful use of thiouracil an operation is avoided, the treatment can in some instances be carried out without putting the patient to bed or even stopping his work, and the thyroid activity can be fairly accurately controlled by carefully regulated

dosage, whereas even the best surgeon has to guess how much of the gland to remove at operation.

On the other hand the cosmetic effect resulting from the surgical removal of a large goitre cannot be achieved by thiouracil, the after treatment is more difficult with thiouracil, and there remains the never-ending possibility of the sudden development of agranulocytosis. In a questionnaire by Winkle et al. (23) addressed in America to 328 clinical investigators who had treated with thiouracil 5,745 patients suffering from hyperthyroidism, the following question among others was asked : " Is the incidence of toxic reactions to thiouracil greater or less than complications of present methods of treating hyperthyroidism ? " The answers showed that 78·6% of the investigators considered it was less, 4·2% that it was greater, and 17·2% expressed no opinion.

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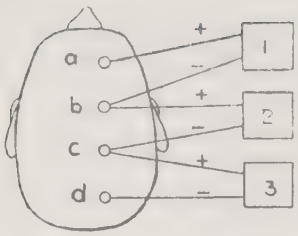
CHAPTER XII

THE NERVOUS SYSTEM

Electro-encephalography

It has been known for over sixty years that electrical currents are produced in the brains of animals both at rest and during stimulation. In 1891 Gotch and Horsley (1) recorded such currents produced in the exposed brains of animals, using a string galvanometer or a capillary electrometer. Berger (2) in 1929 first showed that changes in electrical potential arising in the cerebral cortex could be demonstrated in man with electrodes applied to the scalp. He used originally a Siemen's galvanometer and the deflections were very small. There was at first some doubt as to whether the electrical changes did actually arise in the brain, but in 1934 Adrian and Matthews (3) confirmed Berger's findings and demonstrated that the waves recorded arose in the parieto-occipital region in both sides of the brain, near Vogt's area 19; all extraneous cause of change in potential, such as muscular activity, eye movements and electrode artefacts were excluded. Electro-encephalography is comparable with electro-cardiography in technique, but the potential changes led off from the scalp are often more than a hundred times smaller than those recorded from the body surface in electro-cardiography. Thus the normal scalp potential changes are in the region of 5 to 100 microvolts, but occasionally under abnormal conditions they may rise to 1 millivolt (1 microvolt = 1 millionth of a volt). The electrical potential changes on the brain surface are ten to fifty times greater than those obtained on the scalp.

The Electro-encephalograph. There are many different types of electrode, the most usual ones being either a saline pad, which may be moved about the head at will, or a solder pledget sealed to the scalp with collodion. The second is commonly used for prolonged investigation. The different techniques used have been fully described by Gibbs, Davis and Lennox (4), Kornmüller (5),



PAIRED ELECTRODES

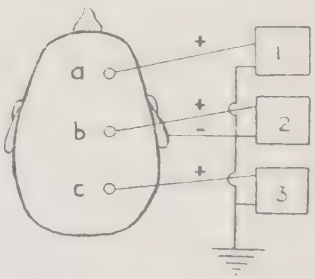
FIG. 33. Paired electrodes (after Williams and Gibbs (8)).

Walter (6), Balado, Romero and Noiseux (7) and others.

The hair should be cut short or pushed aside under the electrodes. It should be moistened if it is very dry, and the portion of scalp under the electrodes should be cleaned with alcohol. Either paired electrodes or " a single scalp electrode system " may be employed. In the paired electrode system four electrodes (a, b, c and d) are placed on the scalp connected with three amplifiers (1, 2 and 3), as shown in the diagram (see Fig. 33). The amplifiers are thermionic valves, whereby an amplification of more than a millionfold can be obtained. The potentials thus amplified are led to three recording oscillographs.

Recording can also be carried out on paper by an ink-writing oscillograph or photographically by means of cathode ray tubes. As the frequencies encountered are relatively slow the ink-writing oscillograph, in virtue of its simplicity and economy of time and money, is superseding the more unstable cathode ray tube.

Single scalp electrodes are employed with an " indifferent " electrode placed on the ear, and earthed. The arrangement of a three single electrode system is shown in the diagram (see Fig. 34); a, b and c are electrodes placed on the scalp and 1, 2 and 3 are amplifiers.



SINGLE ELECTRODES

FIG. 34. Single electrodes (after Williams and Gibbs (8)).

The single scalp electrode records the potential change occurring at a single point in the brain and gives a truer reproduction of a pure wave than do the paired electrodes. It therefore records accurately the abnormal wave forms which are encountered in grand mal, petit mal and psychomotor epilepsy. The paired electrodes are used to localise foci of activity, for with paired electrodes changes in potential common to both electrodes are eliminated, and although a true picture of the electrical events

occurring in the cortex is not given, the local discharges are rendered more prominent. As Williams and Gibbs (8) point out, if the potential gradient is visualised as a hill, the single scalp electrode records the height of the hill above the ground level, represented by the " indifferent " electrode, whereas the paired electrodes measure the difference between the two points on the hill under examination without indicating the actual height of either point above the ground level. Thus with the single scalp electrode system, when the " hill top " or greatest change in potential is placed under one electrode, whichever way the electrode is moved, the potential will fall off as shown by the recorded

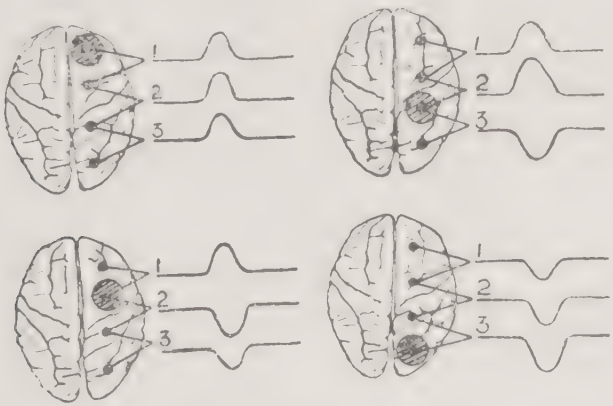


FIG. 35. Localisation of focus of activity by paired electrodes (after Williams and Gibbs (8)).

deflections. With paired electrodes, if the " hill top " is at the end of the row of electrodes the potential will fall in the same direction in each pair and will cause a deflection of each of the recorders in the same direction, the maximum deflection occurring from the electrode situated over the focus of activity. If, however, the " hill top " occurs at one of the middle electrodes the fall will be away from that electrode in both directions, and so will pass through the circuit on each side in opposite directions, making an upward signal or deflection in one, and a downward in the other ; the waves are then said to be " out of phase," indicating that the focus of activity is situated under, or adjacent to, the middle electrode common to the two pairs.

The application of this method for the localisation of the focus

of activity, by means of paired electrodes, is shown in the diagram (see Fig. 35).

The Alpha Waves or Berger Rhythm. Berger, placing one electrode on the frontal and the other on the occipital region, demonstrated the occurrence of waves arising from the brain in health. They have an amplitude of 10 to 100 microvolts and a frequency of about 10 Herz (10 cycles per second). In childhood the frequency is about 6 per second and after the age of fifty the frequency diminishes. They are present when the eyes are shut and usually disappear when the eyes are open. In a few people they are not present. They are nearly sinusoidal and come and go at irregular intervals (see Fig. 36A). When the eyes are shut mental concentration or a sudden noise will cause the wave to diminish in amplitude. Rarely, with sufficient mental relaxation, the waves persist even with the eyes open. The alpha waves are replaced by other wave forms during sleep. They are present in blind people, but noises or tactile stimuli may cause their disappearance. The persistence of the waves therefore appears to depend upon that part of the cerebral cortex which is concerned with the integration of the visual stimuli. They are thought to be produced by groups of neurones acting in unison, their synchronisation being due to a local pace-maker, whose activity is modified when the neurones are brought into action. Thus they are evident when cortical activity is suppressed, and abolished during functional activity. Lemere (9) has used this method to distinguish between blindness and hysterical blindness or malingering. In true blindness the alpha waves persist when the eyes are opened and the person attempts to look at an object; in feigned or hysterical blindness the alpha waves cease on opening the eyes and looking at an object. This suggests that in hysteria neither the sensory nor motor pathways, nor the cortical centres are affected.

The Beta Rhythm. Berger also described beta waves occurring in health. They are smaller than the alpha waves and have a frequency of 18 to 25 per second. They are believed to arise in the pre-frontal region of the cerebral cortex. Similar waves can be produced by contraction of the frontal and temporal muscles, and they are more prominent in records from individuals with wrinkled foreheads and raised eyebrows. Little is known

about these minute waves, and their significance is not yet understood.

Changes in the Electro-encephalogram in Health

Sleep. Loomis, Harvey and Hobart (10) have shown that the alpha waves persist during the preliminary drowsy stage while the subject is still conscious. With the onset of sleep the alpha waves cease and intermittent groups of waves appear, known as "spindles," with a frequency of 14 or 15 per second. Later, in addition to the "spindles," larger and more prolonged waves, known as delta waves are seen. A dream may be accompanied by the appearance of alpha waves.

Hypnosis. The alpha rhythm is sometimes slowed during hypnosis, but the electro-encephalogram does not resemble that of natural sleep.

Cerebral narcotics cause the appearance of slower and larger waves, and basal narcotics result in quicker and larger waves.

The Electro-encephalogram in Disease

Studies have been chiefly directed to changes seen in the electro-encephalogram in increased intracranial pressure, in cerebral tumours, and in the epilepsies.

Increased Intracranial Pressure. This may be due to obstruction of the ventricular system, to cerebral tumours, concussion or meningitis. Berger (11) in 1931 showed that slow delta waves can be detected all over the skull. The waves may have an amplitude up to 100 microvolts and a frequency of 3 per second. Williams (12) has shown that the abnormal waves are not directly related to the raised intracranial pressure, but are caused by changes in the water content of the cerebral hemispheres, which is probably limited to the white matter. If the increased pressure is due to a cerebral tumour, reduction of the pressure by intravenous injection of hypertonic sodium chloride solution may enable the site of the tumour to be located, as the slow waves are now confined to the site of the tumour, as shown by Walter (6).

Cerebral Tumours. In 1933 Berger (13) demonstrated similar slow waves arising over the affected part of the brain in two cases of cerebral tumour. The waves appeared when one of the electrodes was placed over the tumour and they were not present

when the tumour was midway between the electrodes. In 1935 Kornmüller (5) and Foerster and Altenburger (14) demonstrated that the potentials arise in the surrounding cerebral tissue and not

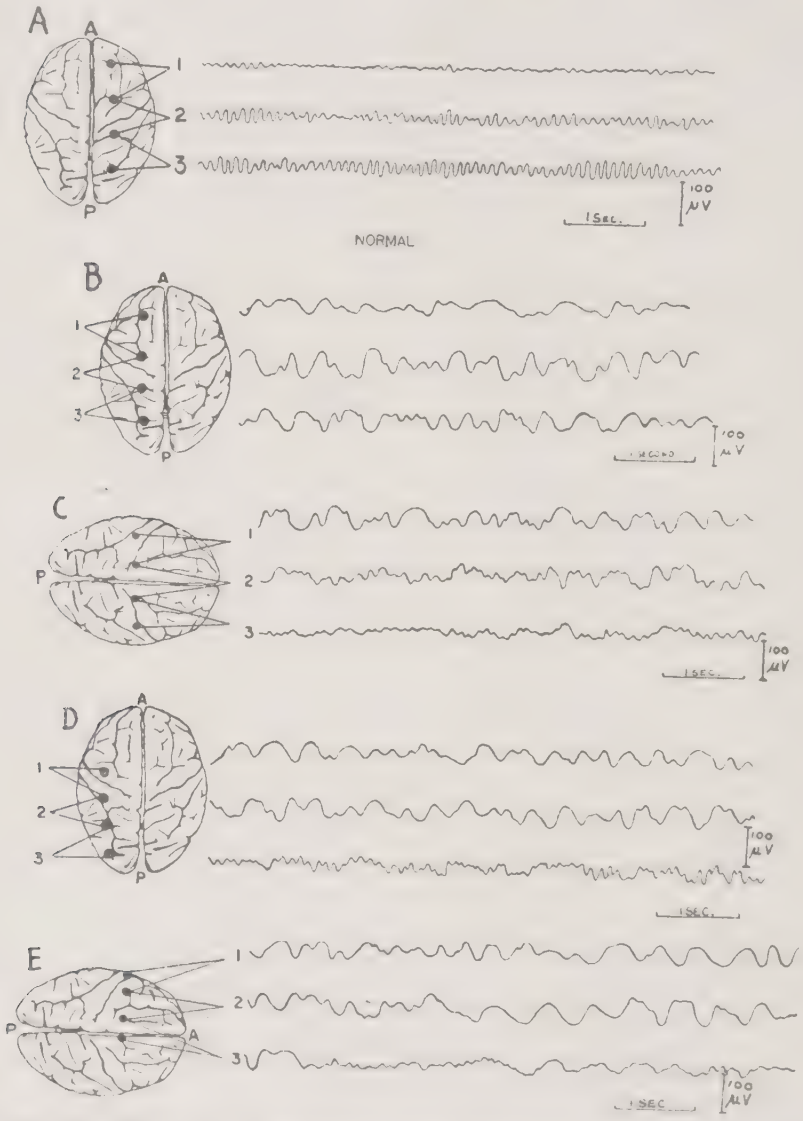


FIG. 36. Method of localisation of a cerebral tumour with paired electrodes (after Williams and Gibbs (8)).

in the tumour itself. Walter (15) has evolved a method for locating certain tumours by means of the electro-encephalogram.

Walter's method of localising a cerebral tumour with paired

electrodes is illustrated in the diagrams (see Fig. 36) reproduced from an article by Williams and Gibbs (8). There are five stages in the investigation : A. Right half of brain normal. B. Abnormal focus in left parietal region. C. Abnormal focus confirmed in left side of brain. D. Abnormal focus localised antero-posteriorly. E. Abnormal focus localised transversely.

Williams and Gibbs (8) investigated 50 cases, in 35 of which the lesion was demonstrated by operation or by autopsy, and in 15 the diagnosis was established by other means. A single focus was found in 36 cases and all were correctly located. In 7 cases two foci were found, in 4 of which the pathological cortical areas were confirmed. In 7 cases generalised abnormal waves were found. In 41 cases of suspected intracranial lesions the electro-encephalogram was normal, and further clinical investigations revealed no abnormality.

In general it may be stated that any tumour causing progressive destruction of cortical tissue will cause abnormally large slow waves of electrical potential to arise in the damaged cortex. Similarly, any other lesion, such as an abscess or a demyelinating disease resulting in cerebral dysfunction, will be associated with identical wave forms. It follows that discrete and non-progressive cortical lesions or deep tumours not affecting the cerebral hemispheres or the cortex itself, or cerebellar tumours are unlikely to be unmasked by indirect electro-encephalography. Walter and Dovey (16) have described a method, using a needle electrode, by which the electrical activity of the subcortical structures can be determined. This can be used to supplement conventional methods of identifying and locating subcortical tumours at operation.

Epilepsy. In 1937 Golla, Graham and Walter (17) showed that there are changes in the electro-encephalogram in 50 per cent. of patients under the age of forty suffering from convulsive epilepsy, even though no fit is seen at the time of examination. The majority of such abnormal electro-encephalograms occurred in patients suffering from grand mal. An abnormal electro-encephalogram is rarely seen in a patient who first has epileptic fits after the age of forty. The abnormality between fits consists in the occurrence of intermittent irregular slow waves. Walter states that in grand mal the focus of discharge is usually found in the region of

the superior frontal gyrus on one or both sides. Krynauw (18) says that foci of discharge are also quite commonly post-central or even occipital in grand mal, and other workers have been unable to find a predilection for any particular cortical area. In petit mal the abnormal waves are widespread but the focus is usually post-central. " Seizure " waves occur during a fit, the focal discharges becoming greater and the area of discharge spreading. The waves are then slower, but larger and more irregular. Transitory bursts of " seizure " waves are often seen in the absence of a fit and Lennox (19) has called them " subclinical seizures." An increase in the number of these outbursts often precedes a grand mal fit by days and can be used in predicting the onset of a convulsion. Gibbs, Gibbs and Lennox (20) describe the " seizure " waves in grand mal as sharp spikes occurring at twenty-five to thirty per second, in petit mal there are quick sharp spikes alternating with slow round waves, and in psycho-motor attacks (psychic variants) square flat waves occur at about three to four per second. As epilepsy is associated with the development of abnormal rhythms in the cerebral cortex they have called it a paroxysmal cerebral dysrhythmia. According to Gibbs (21) about 10% of normal people show a cerebral dysrhythmia indistinguishable from that occurring between the seizures in epilepsy, and it is possible that such individuals are suffering from asymptomatic epilepsy. Further about 5% of the near relatives of epileptics show an abnormal electro-encephalogram. Despite this, corroborative evidence of petit mal provided by an electro-encephalogram was successfully used by the defence in a murder case in England in 1942, when prolonged clinical observation of the accused in prison showed no evidence of epilepsy.

Symptomatic Epilepsy. There is no difference between the record of a fit resulting from trauma, tumour or any other lesion and that seen in idiopathic epilepsy. The fit may not necessarily be associated with any focal electrical abnormality.

Hysterical Fits. No abnormality is seen in the electro-encephalogram during the fits, and, except that unstable people are more liable to show abnormalities in the electro-encephalogram than are stable subjects, the resting record is usually perfectly normal.

Golla, McKissock and Walter (22) have shown that surgical removal of the portion of the cerebral cortex in which the delta

waves originate may abolish the fits for a few months, but they ultimately recur. The actual origin of the fit therefore appears to be due to generalised cerebral changes.

Anti-convulsant drugs, such as bromides and Luminal, which are used for the treatment of epilepsy, reduce the number of abnormal electrical outbursts, or "subclinical fits," especially in grand mal epilepsy.

The types of waves met with in epilepsy are illustrated in the accompanying diagram (see Fig. 37).

Head Injuries. Williams (23) has investigated with the electro-encephalograph 74 patients suffering from acute head injuries due to war wounds. The tests were made within twenty days of the injury. He found that widespread abnormally slow waves, suppression of normal frequencies, and outbursts of high-voltage

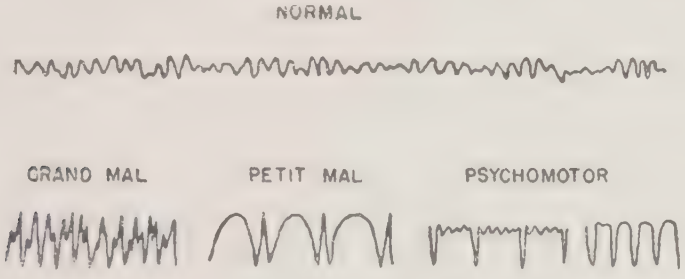


Fig. 37. Types of waves in epilepsy (Lennox (18)).

sine waves with a frequency of 2 or 3 per second, indistinguishable from sub-clinical epileptic attacks, occurred in the acute stage. Further, the degree of abnormality corresponded with the clinical state of the patient, and progressive changes indicated improvement or deterioration. The epileptic type of waves bore no prognostic significance as regards the occurrence of post-traumatic epilepsy. Williams (23) also investigated the electro-encephalograms in chronic post-traumatic states following head injuries. The electro-encephalogram was abnormal in 50% of cases and there was a definite relation between the electro-encephalogram, the severity of the injury, the persistence of symptoms and the presence of dural penetration.

Psychopathic Disorders. Attacks of uncontrollable anger or negativism, and behaviour disorders in children may be accompanied by cerebral dysrhythmia and an abnormal electro-

encephalogram. The electro-encephalogram will also indicate whether the cerebral cortex is affected in cases of Sydenham's chorea.

Conclusions. Electro-encephalography, which started life as suspect, is now accepted as a genuine method of studying electrical changes in the cerebral cortex. It has already become a useful adjunct to other clinical methods in investigating the site of cerebral tumours and abscesses, and it has special value in giving objective proof of the presence of epilepsy and the exact type of fit in subjects whose history is inconclusive. It is of value in demonstrating impairment of function of the central nervous system in cases of head injuries. It is also helpful in the diagnosis of narcolepsy, subdural hæmatoma, cerebral trauma, meningitis, encephalitis, Schilder's disease and disorders of behaviour. Severe degrees of dementia may occur with a normal electro-encephalogram.

The Use of Sodium Diphenyl-Hydantionate in Epilepsy

In 1937 Putnam and Merritt (24), experimenting on the effects of drugs in the prevention of electrically produced convulsions in cats, found that sodium diphenyl-hydantionate was superior to numerous other substances, including bromides and phenobarbitone. Merritt and Putnam (25) subsequently introduced this drug for the treatment of epilepsy in man. It is known in America as Dilantin Sodium and in Great Britain as Epanutin, Eptoin, Solantoin, and Soluble Phenytoin. It is put up in capsules containing 0·1 g. ($1\frac{1}{2}$ gr.) for adults, and 0·05 g. ($\frac{3}{4}$ gr.) for infants and children under six years of age.

Dosage. Merritt and Putnam (25) recommend, as an average dose for an adult, one capsule (0·1 g.) t.d.s. In some cases four to six capsules daily are required. Robinson and Osgood (26) regard six capsules a day as a maximum dose and hesitate to give more than four capsules daily. For children under the age of six years, half the adult dose should be prescribed. Fetterman (27) advises that the change-over from phenobarbitone or other sedative should be made slowly. Thus if the patient was taking three tablets of phenobarbitone daily, one tablet should be replaced by one capsule each week. It may be advisable to continue the

administration of one dose of barbitone with three to five capsules of Epanutin daily. Similarly, the change back from Epanutin to other drugs should be made gradually, otherwise status epilepticus may be precipitated.

The capsules are prescribed before food unless gastric disturbances occur, when they should be taken directly after meals. Williams (28) recommends that if relief is not obtained with five capsules daily the drug should be discontinued or supplemented with other anti-convulsants.

Indications for Epanutin and Results of Treatment. Merritt and Putnam (29) found that Dilantin Sodium was of greatest value in the treatment of grand mal and of the psychomotor (psychic equivalent) types of idiopathic epilepsy, and of less value in petit mal seizures. Thus in 6 cases of psychomotor equivalents there was complete relief in 67%; in 118 cases of grand mal there was complete relief in 58%; and in 74 cases of petit mal complete relief was obtained in 35%. Williams (28) reported on a series of 91 cases of chronic epilepsy (treated at The National Hospital, Queen Square, London). He found that in 79% of cases of grand mal, and 63% of cases of petit mal, the fits were reduced in frequency as compared with their occurrence during other methods of treatment. In 15 cases the improvement was dramatic, but in some instances the initial good effect wore off later. Many reports have now been published on the results of treatment with Epanutin, but they are not universally favourable. Blair (30) has treated 29 male and 7 female patients with Epanutin, of whom 13 male and 7 female received a full year's treatment. They were all chronic epileptic psychotics with an average age of about forty years. The female cases had responded badly to other treatments. Treatment was discontinued in 16 cases on account of exacerbation of fits or toxic symptoms, and in 4 cases death occurred which was not shown to be due to the drug. Of the 20 cases only 1 had complete relief, having no fits during the last six months. Six patients showed a great decrease in fits, *i.e.*, a reduction of more than 75%, 5 cases showed a moderate decrease, *i.e.*, a reduction between 50% and 75% of fits, and 2 showed a slight decrease, *i.e.*, a reduction of between 25% and 50% of fits. Five showed no change and 1 was worse. In every case except 1, reduction in fits refers to grand mal seizures. Tullidge and

Fox (31) treated 63 adolescents with Soluble Phenytoin. In 20 cases the drug could not be given for more than two months owing to toxic effects. The effective therapeutic dose was found to be very near to the toxic dose. In the 43 cases so treated with the effective dose 24 were completely, and 16 almost completely, free from fits. They were all cases which had proved resistant to previous medication.

The use of Epanutin, as opposed to phenobarbitone, results in the patient becoming more alert and wakeful. A gain of weight during treatment is a favourable sign.

Toxic Effects. These are comparatively common, as there is only a narrow margin between the therapeutic and toxic dose of the drug. They are often noted during the second week of treatment, but may occur earlier or later. The toxic effects may be grouped according to the system affected as follows .—

Alimentary. Nausea, vomiting and constipation. Kimball (32) noted a firm painless hypertrophy of the gums, associated with a low vitamin C content of the blood, in 68 patients out of 119 treated with Dilantin. The condition, however, is probably not due to scurvy. The gums do not bleed easily. Gruhzit (33) further showed that in animals Dilantin does not interfere with the absorption of vitamin C, and Pratt (34) found a mild degree of hyperplasia of the gums in 50% of cases receiving Dilantin treatment, and in a similar number of control cases not receiving Dilantin. Hyperplasia of the gums seems much more common in America than in England, and Blair (30) did not observe it in any of his cases. It does, however, appear advisable that dental sepsis should be treated before giving Epanutin.

Nervous. Ataxia may be severe, the patient rolling about, the condition suggesting a cerebellar lesion. There may also be nystagmus, tremors and slurred speech. Other nervous symptoms include diplopia, ptosis, blurred vision, headache, insomnia, restlessness, irritability, hallucinations, delusions, euphoria or a reactivation of suicidal tendencies.

Cutaneous. Various rashes may occur, erythematous, morbilliform or scarlatiniform. A severe exfoliative dermatitis with fever, leucocytosis and eosinophilia has also been noted. An increased growth of hair on the arms and face has been observed in some female patients.

Hæmopoietic. An eosinophilia of from 5 to 12% has been occasionally noted. A non-thrombocytopenic purpura was observed in one case by Merritt and Putnam (25). Epistaxis associated with a rise of blood pressure has been noted by Frost (35).

Treatment of Toxic Symptoms. The Epanutin should be discontinued as soon as any toxic symptom is observed. In the event of an excessive number of seizures following the withdrawal of the drug, an intramuscular injection of Luminal, gr. ½ to 3, should be given, followed by a return to the anti-convulsant previously used. If the toxic symptoms are severe, or if the patient has not responded to Epanutin better than to other forms of treatment, it should not be given again. If, however, the toxic symptoms are slight and disappear in a day or so, Epanutin may be given again in a smaller dose, *e.g.*, one capsule less a day. If there is nausea and vomiting, the administration of acid hydrochlor. dil. m. 15 in water before meals, the Epanutin being given directly after meals, will sometimes enable the drug to be continued. Black (36) and Lennox (37) advise that Benzedrine sulphate (amphetamina sulphas), 5 to 10 mg., be given during the first half of the day to relieve the sense of weariness of which the patient may complain. Larger doses of Epanutin may thus sometimes be tolerated.

Conclusions. Epanutin is a powerful anti-convulsant, and a useful addition to the drugs used for the treatment of idiopathic epilepsy, but it is not without danger. The patient must be under very careful medical supervision during the first few weeks of its administration. It should only be tried when treatment with other drugs, such as bromides, phenobarbitone, belladonna and borax have failed. If Epanutin alone fails it should be tried in combination with phenobarbitone or bromides. It appears to be more efficacious in grand mal than in petit mal. In some cases, although there is a reduction of major attacks there is an increase in the minor ones. In others mental confusion may result, although there has been prolonged freedom from fits. Great care should be exercised in the administration of the drug to the aged or debilitated. In no case can the drug be regarded as a cure for epilepsy. Its administration has to be continued indefinitely and the dose cannot be reduced.

Thymectomy in Myasthenia Gravis

In 1901 Weigert (38) recorded a case of myasthenia gravis with hyperplasia of the thymus. Since then numerous observations have been made and it appears that the thymus is always persistent, hypertrophied, or the seat of a benign tumour in myasthenia gravis. Norris (39) suggests that in myasthenia gravis the thymus shows specific histological changes, consisting of hyperplasia of the epithelial cells of the medulla. In the hyperplastic areas the normal structure of the gland may be preserved, or an undifferentiated syncytial mass of epithelium is found obliterating the round cells and Hassall's corpuscles, its structure closely resembling that of the fœtal thymus. The causal connection, if any, between changes in the thymus and myasthenia gravis is unknown, but it has been suggested that a substance is formed in the thymus which inhibits the cholinergic transmission from nerve to muscle. Sauerbruch (40) in 1912 introduced the operation of thymectomy for myasthenia gravis, the patient was also suffering from hyperthyroidism. Blalock et al. (41) in 1941 reported on thymectomy in 6 cases of myasthenia gravis. One patient died, 3 were symptom-free three months after operation, one was improved but still required prostigmin, and one was unchanged two months later. Carson and Keynes (42) treated 12 patients of from eighteen to forty-four years of age with symptoms extending over seven months to ten years. All were severe cases and unable to work. Three suffered from thyrotoxicosis as well. Three patients died after operation. Three were completely cured nine, six and three months after operation, and returned to work. Three were improved; one of these had previously had deep X-ray treatment to the thymus with acute exacerbation of symptoms, but later improved. After operation she further improved, but still required prostigmin; another of these was also thyrotoxic, and thyroidectomy increased the myasthenic symptoms. Subsequent thymectomy considerably improved his condition, but he still required small doses of prostigmin; the third case was much improved, but also required small doses of prostigmin. In one case there was only slight improvement post-operatively. Two cases were unchanged. Brain and Galloway (43) treated 3 cases by thymectomy without definite improvement.

Keynes (44) has later reported on 25 cases of total thymectomy, and says the results are very erratic, and it is misleading to publish single failures or single successes as is sometimes done. If improvement occurs, it is most marked in a few days after the operation and there is further improvement in the next six to eight weeks. It is usually considered advisable that the mediastinum should be fully exposed by splitting the sternum as far down as the fifth rib, in order to remove all the thymic tissue, and great care is necessary to avoid opening the pleural cavities. Whether any better results are obtained by thymectomy than by deep X-ray treatment of the thymus is undecided. From time to time successful treatment by the latter method is reported. However, as spontaneous remission may occur in myasthenia it is difficult to judge the efficacy of any form of therapy.

Conclusion. Thymectomy is only to be considered in very severe cases of myasthenia gravis, uncontrolled by prostigmin, and then should be carried out by a skilled chest surgeon.

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CHAPTER XIII

THE HÆMOPOIETIC SYSTEM

Types of Anæmia and their Treatment

As our knowledge of the causation of the various anæmias increases, ætiological classifications become more satisfactory. The following classification is based on that of Davidson (1).

1. *Nutritional Deficiency Anæmias.* These may be due to: (*a*) Lack of the anti-anæmic or hæmopoietic principle. This is a primary defect (the diet being adequate) in pernicious anæmia, and a secondary defect (*i.e.*, resulting from a recognisable cause) in the anæmias of sprue and of dysentery, in tropical megalocytic anæmia, in the "pernicious anæmia of pregnancy," in Diphyllobothrium latum infestation, in carcinoma of the stomach and after gastrectomy. In the latter group there is also often an error in iron assimilation. There may be a deficiency of storage of the hæmopoietic factor in advanced cirrhosis of the liver. (*b*) Lack of the hæmopoietic factors, such as iron, copper, thyroxine, vitamin C and a proper salt balance in the food. This deficiency may be primary, as in simple achlorhydric anæmia and the Plummer-Vinson syndrome in which the food intake is often satisfactory but the absence of hydrochloric acid results in impaired absorption of iron, or secondary to a recognisable food or endocrine defect, as in the simple nutritional anæmia of infants, chlorosis, chronic gastritis and enteritis, cœliac disease, starvation, prolonged milk feeding as in the treatment of peptic ulcers, and in myxœdema.

2. *Post-hæmorrhagic Anæmias.* These occur after acute or chronic hæmorrhage.

3. *Hæmolytic Anæmias.* (*a*) Acute hæmolysis may result from blackwater fever, malaria, paroxysmal hæmoglobinuria, septicæmia, or toxins such as snake venoms. It also occurs in Lederer's anæmia. (*b*) A persistent hæmolysis, as in congenital or acquired acholuric jaundice, sickle-cell anæmia, Cooley's anæmia, Von Jaksch's anæmia and lead poisoning.

4. *Anæmias due to Inhibition of the Bone Marrow Function.* These may be primary, as in aplastic anæmia, or secondary, as in aplastic anæmia due to agents such as X-rays, radium, benzol,

lead or mercury. A leuco-erythroblastic anæmia results from involvement of the bone marrow in carcinomatosis, myelomatosis, Hodgkin's disease and osteosclerosis. By some writers Groups 1 and 4 in the above classification are called the dyshæmopoietic anæmias.

An alternative classification is based on the size of the red cells and their hæmoglobin content. This is of great clinical value, as it emphasises the fact that in general the macrocytic anæmias respond to liver and the hypochromic anæmias to iron therapy. Iron is, however, required in addition to liver for macrocytic anæmias if the saturation index is low. The following classification follows that of Wintrobe (2) :—

1. *Macrocytic Anæmias.* In this group the average size of the red cell is increased. The mean corpuscular volume is greater than 94 cμ. The group includes pernicious anæmia, achrestic anæmia, the anæmia associated with liver disorders and with myxœdema, the anæmia of sprue, the " pernicious anæmia of pregnancy," the anæmia of chylous diarrhœa and of Diphyllobothrium latum infestation.

2. *Normocytic Anæmias.* Here the average size of the red cell is normal. The mean corpuscular volume is between 78 and 94 cμ. The cells may be normochromic or hypochromic. The anæmias included are aplastic anæmia, the anæmia following an acute hæmorrhage and that due to malaria.

3. *Simple Microcytic Anæmias.* There is a slight decrease in the size of the red cells. The mean corpuscular volume is less than 78 cμ. The cells are normochromic. Such anæmias may occur in chronic infections and in carcinoma.

4. *Microcytic Hypochromic Anæmias.* The red cells are smaller than normal and their hæmoglobin content is considerably reduced. The mean corpuscular concentration is less than 32%. Examples of anæmias of this class are simple achlorhydric anæmia, the anæmia of the Plummer-Vinson syndrome, the anæmia resulting from chronic hæmorrhage or from ankylostomiasis, the simple nutritional anæmia of infants and chlorosis.

The Macrocytic Anæmias

Pernicious Anæmia. The use of liver in the treatment of pernicious anæmia constitutes a therapeutic advance of similar

importance to the discovery of insulin for the treatment of diabetes. There is little doubt that the origin of the idea can be found in the researches of Whipple (3) and his collaborators. These workers produced a severe secondary anæmia in dogs by bleeding, and proceeded to study the effects of various food substances on blood regeneration. They found that if liver were added in large quantities to the diet, there was a remarkable increase in the blood count which appeared to be entirely due to the liver. Shortly after the publication of these results, Minot and Murphy (4) described the application of the same principles to the treatment of pernicious anæmia. In 1926 they reported the effect of a diet rich in liver in forty-five cases of pernicious anæmia. These results were very striking indeed, since they showed that one month's treatment increased the blood count by about 2,000,000 red cells. A series of publications followed, with the result that the regimen was fully upheld, and now experience has proved that the blood picture can be restored almost to normal in practically all patients.

Castle and his colleagues of Boston (5, 6) by means of a large number of experiments on the action of healthy gastric juice on beef muscle, have been able to show that the juice of the healthy stomach contains some unknown ingredient (intrinsic factor) which, through its action on an extrinsic factor contained in muscle protein, results in the formation of the anti-anæmic or hæmopoietic substance. This is able to produce a remission in pernicious anæmia equal in degree to that produced by liver extract. The identity of the intrinsic factor has not been established, but it is believed that it is quite unrelated to HCl or pepsin, and the work of Taylor et al. (7) suggests that it is probably a ferment. Formijne (8) concludes that no reaction can occur *in vitro* between the intrinsic and extrinsic factors, and he suggests that the reaction occurs in the intestinal wall. It is thermolabile and capable of acting in a " neutral " medium. These researches established the ætiological importance of the stomach and its secretion in the development of pernicious anæmia. The anti-anæmic substance, thus formed, is absorbed from the intestine and stored in the liver and to a lesser degree in the kidneys and spleen. In pernicious anæmia the intrinsic factor is absent from the gastric juice and achylia gastrica is usually present. Cohn

et al. (9) have shown that the active principle is neither protein, lipoid nor carbohydrate, but is either a nitrogenous base or a polypeptide.

The Source of the Intrinsic Factor. Meulengracht (10) using the pig's stomach, found that preparations from the pyloric gland region were highly active as regards the presence of the anti-anæmic factor, whereas those from the fundus gland region were inactive. It therefore appeared probable that the "intrinsic factor" is connected with, or produced by the pyloric glands. Anti-anæmic activity was also demonstrated in preparations from the duodenum, and it was considered that this was due to Brunner's glands, which are histologically identical with the pyloric glands. The two sets of glands are called the pyloric gland organ. The cells in the fundus of the stomach which produce hydrochloric acid and pepsin would not appear from these findings to be concerned with the production of the intrinsic factor. It has been known for many years that there is atrophy of the gastric mucous membrane in pernicious anæmia, and it therefore appeared probable that the atrophy would affect the cells which produce the intrinsic factor. Almost simultaneously investigations on this point were carried out by Meulengracht (11) and by Magnus and Ungley (12). Meulengracht examined histologically the stomach and duodenum in 8 cases of pernicious anæmia, and to his surprise found that although changes were present in the fundus portion, with atrophy of glands and disappearance of the parietal and chief cells in all the cases, the glands in the pyloric region seemed relatively well preserved and no histological changes could be demonstrated in Brunner's glands. Magnus and Ungley examined the stomach from 7 cases of pernicious anæmia. In all a characteristic non-inflammatory atrophic lesion was found, localised to the region of the body mucosa, which did not affect the pyloro-duodenal region. The work of Fox and Castle (13) appears to explain this anomaly. Extracts were made from the human stomach shortly after death, and it was shown that those areas containing the fundus type of cell yielded a substance highly active in hæmopoietic properties, whereas the pyloric gland organ extracts were inactive. Man's stomach differs therefore in this respect from that of the pig. If this is so, the findings of Schenken et al. (14) are contradictory. They prepared liver

extracts in cases of carcinoma of the stomach in man, and determined their anti-anæmic properties. When the carcinoma involved all the stomach except the pyloric portion, the liver extract was potent. When the pyloric portion was affected, the liver extract did not contain the anti-anæmic principle. Goldhammer (15) has shown that the volume of gastric juice is reduced in pernicious anæmia, from a normal of 150 ml. per hour to 20 ml. per hour in pernicious anæmia. The anti-anæmic activity per unit volume, however, was the same in pernicious anæmia as in health. This observation supports the view that the change in pernicious anæmia is a quantitative rather than a qualitative one.

Very little can be stated with regard to the mode of action of liver extract. The changes produced in the blood as the result of successful treatment include the restoration of a normal red cell count, return of the colour index to normal, and an increase in the number of white cells with disappearance of the relative lymphocytosis. Special attention is directed to the reticulocytes present in the blood. These are immature red cells, normally present in the blood to the extent of 0·1 to 1% of the red cells. They cannot be seen in dried stained films, but are demonstrated by " vital " staining in wet films. A blood film is made on a slide, which has previously been coated with a thin dried film of a dye, such as cresyl-blue. A cover slip is then placed on the blood film, which is examined wet (Davidson et al. (16)). The reticulocytes contain a network, granules or dots, stained blue. They probably correspond with the red cells showing polychromasia in the ordinary dry films. In untreated pernicious anæmia the reticulocytes are usually somewhat increased in number, as 2% or more of the red cells. In cases of pernicious anæmia treated with liver, in which a good response can be expected, the reticulocytes rise rapidly to 15 or 30% in a week, and then fall suddenly (reticulocyte crisis) to reach normal in about three weeks. The increase in the red cell count begins usually about the time of the reticulocyte crisis, and persists for several weeks after. The excess of bilirubin in the serum disappears early, at the time of the rise in the reticulocyte count, as shown by Dyke and Greener (17). An increase in the eosinophils after the administration of raw liver has been noted by Whitby (18) and other workers; this usually

reaches a maximum, which may be as high as 26%, between the eighth and twelfth weeks of treatment. Achlorhydria persists after liver treatment.

Stomach Preparations. Following the initial papers of Castle, Sturgis and Isaacs (19) published their observations on the anti-anæmic principle contained in dried whole hog's stomach. The indications for its use are the same as those for liver and liver extract. Accounts have been published in which stomach extract has produced a remission where liver has failed. Thus Renshaw (20) describes a patient refractory to liver and liver extract, but responding readily to stomach extract. No definite explanation has been offered for their action, but the most ingenious is that of Castle, who suggests that the autolysis which occurs after death results in the interaction of the healthy gastric juice with the muscle protein of the gastric muscularis, and that by this means the necessary factor is formed. Wilkinson (21) showed that the mucous membrane and the muscle layer of the hog's stomach yield a light powder when desiccated at 40° C. *in vacuo.* Preparations from both sources are effective in the treatment of pernicious anæmia, and weight for weight are equal to, or better than, a liver diet. He also showed that normal human gastric juice, when given during meals to a patient suffering from pernicious anæmia, causes improvement. Later, Wilkinson and Klein (22) demonstrated that an active gastric extract can also be obtained by expression of the stomach juice, using a pressure of 2½ to 3 tons per square inch and subsequently treating the juice with alcohol. This active principle they termed hæmopoietin. They regard this as the same as, or closely allied to, Castle's intrinsic factor. Wilkinson and Klein (23) further showed that when hæmopoietin is allowed to act on beef outside the body a relatively heat-resistant substance is obtained which resembles the liver active principle. Wilkinson (24) considered hæmopoietin to be an enzyme-like substance which is very easily destroyed by heat and which requires a substrate for its action. This hæmopoietin is only found in the stomach of carnivoræ.

Yeast Preparations. The beneficial effect of marmite in the treatment of certain megalocytic anæmias requires consideration and the facts observed may be briefly stated. Marmite is a yeast extract, rich in the vitamin B complex, containing the antineuritic

vitamin B_1, and nicotinic acid. Wills (25), in India, found that marmite was as active as liver in causing regeneration of cells in the "pernicious anæmia of pregnancy" and in tropical macrocytic anæmia. Wills and Evans (26) have subsequently shown that tropical macrocytic anæmia does not respond to the highly purified liver extracts which cure pernicious anæmia. It does respond, however, to crude liver extract and to autolysed yeast extracts. The active factor is not vitamin B_1, B_4, riboflavin or nicotinic acid, but it may be the extrinsic factor. It is now generally agreed, as Davidson (27) stated, that marmite alone will not cure pernicious anæmia during the acute stage. He states that many cases in the relapse stage fail to respond to vitamin B, and that marmite cannot replace liver or hog's stomach extract. Marmite is efficacious in the treatment of tropical macrocytic anæmia, the anæmia of sprue and of cœliac disease. In these diseases, however, there is no achlorhydria and the stomach produces the "intrinsic factor," which acts on the "extrinsic factor" to form the anti-anæmic substance. In those cases of pernicious anæmia in which a response to marmite is obtained there is possibly a small amount of the "intrinsic factor" present in the gastric juice. In subacute combined degeneration of the cord associated with pernicious anæmia, marmite is not of value.

Treatment of Pernicious Anæmia

In a very severe case a preliminary transfusion of 500 ml. of compatible blood given slowly is of great value, if due care is taken in matching the blood.

Parenteral Administration of Liver. In addition, an intravenous injection of a liver extract, such as Neo-Hepatex or Hepastab Forte, 5 ml., should be administered slowly, or 6 to 8 ml. of Neo-Hepatex or Hepastab given intramuscularly the first day, and 2 to 4 ml. on the following days, until a reticulocyte response is obtained. For subsequent treatment a preparation such as Neo-Hepatex or Hepastab is injected intramuscularly. The maintenance dose is 2 to 4 ml. weekly until the blood is normal and there are no symptoms. Later, 2 to 4 ml. are required every two, three or even six weeks.

In 1935 Dakin and West (28) obtained the anti-anæmic principle of liver in a still more concentrated state. It is known as

Anahæmin and is put up in ampoules containing 100 mg. in 1 ml. The initial dose for a severe case of pernicious anæmia is 0·5 ml., repeated daily for a week, or a single intramuscular injection of 4 ml. may be given. Subsequently a maintenance dose of 2 ml. is injected every two to four weeks. Examen or Hepastab Forte may be used instead, the dose being approximately double that of Anahæmin. Proteolysed liver was prepared in 1943 by Davis et al. (29) by digesting minced liver with papain, a proteolytic enzyme which reacts at the natural *p*H of minced liver, approximately 5·6. The product is put up as a dry powder, freely soluble in cold water. It is administered dissolved in warm water flavoured with pepper and salt, the dose being 2 dr. to 1 oz. daily. It has been found of value in cases of megaloblastic anæmia refractory to the parenteral administration of liver, including idiopathic cases and those associated with pregnancy, sprue, and the puerperium.

Administration of Iron and Vitamin C. If the mean corpuscular hæmoglobin is below 32%, iron should be given by mouth, in the form of ferri et ammon. cit. gr. 30 t.i.d., in addition to the liver treatment. Paræsthetic symptoms may be relieved by the iron. In some cases of refractory pernicious anæmia, as described by Wilkinson (30), ascorbic acid, 100 to 150 mg. must be given by mouth in addition to the liver in order to obtain a remission of the disease.

Oral Administration of Stomach Preparations. These are made as described above by desiccation, and are not extracts. Wilkinson (31), in 1931, showed that patients suffering from pernicious anæmia rapidly improved with 250 g. of fresh uncooked mucous membrane of hog's stomach daily. Desiccated preparations of whole hog's stomach are also available, such as Pepsac or Gastrosic. Twenty-five grams are equivalent to 100 g. of fresh whole hog's stomach. The usual dose is 10 g. three times a day. The powder must be given cold, either in soda water, cold milk, oxo, bovril, port or burgundy. Wilkinson claims that this treatment is superior to liver treatment in the speed of remission, the relief of gastro-intestinal symptoms and in the rate at which normal health is regained. Thus Wilkinson (32) has found that in seven to twelve days the reticulocytes reach a peak of 30 to 70%, followed by a rapid rise of the blood count. The maintenance dose

is up to 28 g. of Pepsac daily. The stomach treatment is considerably cheaper than liver treatment. In conclusion, there are three essentials in the use of stomach preparations : (1) The preparation must be active. (2) It must be free from micro-organisms. Neglect of this simple precaution has resulted in the death of the patient. (3) The preparation must be given cold.

Parenteral administration of stomach preparations is not possible.

The Treatment of Nervous Lesions. Ungley (33) recommends that in cases of spinal cord degeneration liver extract should be injected in doses many times greater than that required for the relief of the anæmia. Highly purified liver products, such as Anahæmin, should not be used as they are ineffective. Sargant and Langmaid (34) obtained good results in the treatment of nervous symptoms in pernicious anæmia by the use of massive doses of iron, such as Blaud's pill in powder form in capsules, 150 grains daily, liver treatment having failed to stop the progress of the nerve lesions. Wilkinson (32) states that patients who have been bedridden owing to postero-lateral sclerosis of the spinal cord have so improved with stomach preparations that they have been able to return to work.

The Prognosis in Pernicious Anæmia. The immediate prognosis in pernicious anæmia has been revolutionised by the introduction of liver therapy. Previous to 1926 the mortality rate was nearly 100%, and death occurred in periods up to two and a half years after the diagnosis was made. Now it is very rare for a patient to die during the acute stage if adequate treatment is given. In every case the correct maintenance dose of liver or stomach preparation must be determined and controlled by regular blood examinations. The position is comparable with the administration of insulin and its control by blood sugar readings. Witts (35) has emphasised the importance of adequate after-treatment in cases of pernicious anæmia, as every patient is under the threat of subacute combined degeneration of the cord. The maintenance dose, either of a liver or stomach preparation, must be sufficient to keep the hæmoglobin over 90% and the red cells over 4,000,000 per c.mm. The blood should be examined every month. The advantage of giving intramuscular injections of liver extract every two to four weeks is that the patient is compelled to remain under medical

supervision. Wilkinson (32) has found that in his series of 404 cases treated since 1929, over 95% have remained well for periods up to five and a half years after discharge from hospital, taking a maintenance dose of desiccated stomach up to 28 g. daily. Hill (36) has drawn attention to the fact that the death rate from pernicious anæmia fell substantially in 1928 following the introduction of liver treatment into England in 1927. Since 1931 there has, however, been a rise again in the death rate. This may be due to inadequate after-treatment, to the disease becoming refractory to treatment after a time, or to patients dying from other diseases than pernicious anæmia, especially intercurrent infections, *i.e.*, to faulty certification.

Achrestic Anæmia (*χρῆσθαι* = to utilise). In 1935 Wilkinson and Israëls (37) described a type of severe macrocytic anæmia which in some respects resembles pernicious anæmia, but is resistant to liver therapy. They considered that it is due "to inability to utilise, or possibly mobilise from the storage depots (such as liver, kidney, brain), the anti-anæmic principle responsible for the correct maturation of megaloblasts; although such may be present in the body in adequate quantities." Clinically it differs from pernicious anæmia in that the age of onset is between early adult life and old age, the sexes are equally affected, the gastric acidity is normal or almost normal, there is no disturbance of the gastro-intestinal tract or central nervous system, and no pyrexia or evidence of hæmolysis. The liver and spleen are not usually enlarged. Examination of the liver in one case showed that the anti-anæmic principle was present in sufficient amount to produce a reticulocyte crisis in a patient suffering from true pernicious anæmia. It was found in about 1% of cases thought to be suffering from pernicious anæmia. Achrestic anæmia has not met with universal acceptance; thus some workers such as Vaughan (38) suggested that it is a megalocytic anæmia associated with liver disease, and others such as Castle and Minot (39), Zanaty (40) and Davidson and Fullerton (41) have pointed out difficulties in differentiating it from aplastic anæmia. Mahler and Greenberg (42) have reported a case of hyperchromic macrocytic anæmia refractory to liver extract, in which they say the weight of evidence strongly favours achrestic anæmia as described by Israëls and Wilkinson. Israëls and Wilkinson (43) after a further study of

six new cases have strengthened their views as to the existence and nature of achrestic anæmia. They say that the only changes in the liver are fatty ones associated with a severe anæmia, and study of the bone marrow differentiates it from aplastic anæmia (see p. 338). One case of achrestic anæmia with achlorhydria was found in 1,100 cases thought to be suffering from pernicious anæmia. Bomfort and Rhoads (44) are inclined to believe that achrestic anæmia is not a specific syndrome.

Treatment. Life may be prolonged by repeated blood transfusions and by intensive anti-anæmic treatment. Liver extract, 2 ml., should be given intramuscularly t.i.d. for ten to fourteen days. Subsequently 4 to 6 ml. should be injected weekly. In addition a stomach preparation such as Pepsac, 1 oz., should be given daily by mouth. Despite treatment the disease appears to be inevitably fatal.

Macrocytic Anæmia associated with Hepatic Disorders. Wintrobe and Shumacker (45) found eleven instances of macrocytic hyperchromic anæmia among 43 cases of hepatic disorders, which included such conditions as cirrhosis, passive congestion, carcinoma and acute yellow atrophy. Free hydrochloric acid was present in the gastric juice of 6 out of 10 cases examined. The anæmia was not severe and remissions are likely to occur apart from treatment. The authors suggest that the anæmia is possibly due to impairment of storage of the hæmopoietic principle in the liver, or to a combination of partial gastric disturbance and incomplete liver damage. Rosenberg (46) investigated 48 cases of cirrhosis of the liver, mostly of an advanced type. In 43 cases there was macrocytosis, but in only 8 was the red cell count below 2·5 millions.

Macrocytic Anæmias in Myxœdema. These are of three types, simple macrocytic normochromic, Addisonian macrocytic normochromic, and macrocytic or normocytic hypochromic anæmia. In other cases of myxœdema a hypochromic or normochromic anæmia is found. Anæmia of some type is present in about 50% of all cases of myxœdema. The simple macrocytic hyperchromic anæmia can be cured by the administration of thyroid alone, as was shown by Bomford (47). The anæmia is never severe, but it may take several months to effect a cure. The Addisonian type of anæmia requires thyroid and liver extract, and the hypochromic anæmia is curable with thyroid and iron.

Tropical Macrocytic Anæmia. The Anæmia of Sprue. The Anæmia of Cœliac Disease. These anæmias are probably nutritional in origin, the gastric secretion being normal. Response to treatment therefore will occur either with liver extract or with marmite. Thus Vaughan and Hunter (48) have recorded good results with marmite in the megalocytic anæmia occurring in cœliac disease.

The Nutritional Megalocytic Anæmia of Pellagra. This has been studied by Moore and his co-workers (49). The diets of their 25 patients had been very deficient in animal protein and the vitamin B complex. Free HCl was present in the gastric juice in the majority of cases, and from their experiments Moore concluded that the anæmia was not due to a deficiency of the intrinsic factor, but to a prolonged lack of Castle's extrinsic factor in the diet, associated in many cases with poor absorption from the intestines. Macrocytic anæmia due to food deficiency can therefore occur without avitaminosis.

The "Pernicious Anæmia of Pregnancy." Several types may occur in association with pregnancy and the puerperium. Thus pregnancy may complicate true Addisonian pernicious anæmia. Osler (50) in 1919 described a "severe anæmia of pregnancy," and this is of a pernicious type. It is more common in India than in temperate zones, and usually shows itself between the sixth and eighth months of pregnancy. As described by Whitby (51) it may be plastic or hypoplastic in type. In the former case there is megalocytosis, with a high colour index and signs of blood regeneration, but in the hypoplastic or aplastic variety there is no sign of regeneration of erythrocytes, the colour index is high, and there is megalocytosis or normocytosis. A chlorotic type of anæmia, due to deficient iron supply, may also complicate pregnancy. Here the colour index is low and there is no megalocytosis. The "pernicious anæmia of pregnancy" differs from Addisonian pernicious anæmia in several respects. Thus achlorhydria is usually absent and there is a tendency to spontaneous recovery after the pregnancy is terminated. Clinically the patient has usually been in good health and there is no evidence of infection. The patient becomes deadly pale, with severe dyspnœa and often œdema or pyrexia. Hæmorrhage may occur from the nose, gastro-intestinal tract or vagina. The condition may be mistaken

for an internal hæmorrhage. Davidson et al. (52) have investigated 16 cases of severe megalocytic anæmia occurring during pregnancy or the puerperium. The colour index may be below unity. Some of these were refractory to treatment by liver extract, iron and yeast, and in such cases life has to be maintained by blood transfusions until the hæmatinic treatment takes effect.

Treatment. In very severe cases blood transfusion of 500 ml. should be given at short intervals, followed by the daily administration of liver, by mouth or injection, or of stomach extract by mouth, 1 oz. daily. This treatment should be continued until the pregnancy is terminated and the anæmia is cured.

The Normocytic Anæmias

Aplastic Anæmia. In this disease there is a progressive failure of function of bone marrow. The anæmia is progressive without remissions. The colour index is usually between 0·9 and 1. There are no reticulocytes. The total white cell count is low, about 1,000, and the polymorphonuclear cells are much reduced. There is no achlorhydria.

Treatment. Liver is not effective. Blood transfusions are the most hopeful line of treatment and the use of concentrated red cell suspensions, the preparation of which is described by MacQuaide and Mollison (53), and Williams and Davie (54), avoids an undue increase of the blood volume.

The Microcytic Hypochromic Anæmias

Simple Achlorhydric Anæmia (Idiopathic microcytic anæmia. Essential hypochromic anæmia). This condition was described by Faber (55) in 1914 and attention has been especially directed to it in England by Witts (56), Hartfall and Witts (57) and Dan Davies (58), and in America by Damashek (59). It affects particularly women between the ages of thirty-five and fifty, the chief symptoms being those of flatulent dyspepsia and dyspnœa. The tongue may be smooth and red and the nails brittle or concave (koilonychia). The dietary has usually been lacking in meat and green vegetables for some time, and owing to the achlorhydria iron absorption is deficient. The average amount of iron in a normal

diet is 10 to 20 mg., of which 7 to 9 mg. are available. There is a daily loss of 5 to 20 mg. in the fæces, and 0·5 to 1 mg. in the urine.

The blood. The red cells may number 4 millions per c.mm. with a hæmoglobin percentage of only 40. Frequently there is microcytosis. There is no increase of bilirubin in the blood.

The stomach. The gastric juice contains mucus in excess and an absence of free hydrochloric acid in 70 to 80% of cases. The stomach empties rapidly or in normal time. Injection of histamine provokes some secretion of hydrochloric acid in from 15 to 25% of cases showing achlorhydria. Further, the gastric juice is not deficient in the " intrinsic factor " of Castle, for on incubation of the juice with beef protein the anti-anæmic principle is developed which is curative of pernicious anæmia. Hartfall and Witts (57) found the " intrinsic factor " below normal in some cases of simple achlorhydric anæmia, but it has not been conclusively shown to be completely absent in any case. This probably explains why the patient does not develop pernicious anæmia, although Damashek (59) suggests that the gastric juice is deficient in the power to digest organic iron.

Treatment. Iron should be given in large doses such as Ferri et ammon. cit. gr. 30, aq. chlorof. ad fl. oz. 1. Fl. oz. 1 t.d.s. In about six or eight weeks the hæmoglobin figure is usually normal. In some cases a mixture containing Acid. hydrochlor. dil. m. 60, tnc. aurantii m. 10, aquam ad m. 120, in a glass of water t.d.s. with meals, relieves the digestive symptoms. The ferri et ammon. cit. may produce diarrhœa, in which case Blaud's pill should be substituted in doses of gr. 15 t.d.s., or Tab. Fersolate, one, t.d.s.

The Nutritional Anæmia of Infants. Mackay and Goodfellow (60) have studied this subject in the hospital type of infant in London. They estimate that 51% of artificially fed babies and 45% of breast-fed babies are anæmic (taking as the standard of anæmia a Hb. figure 10% below normal). They consider this is due to a deficiency of iron and mineral substance in the food.

Treatment. This should be started before the age of two months, as a prophylactic measure. A mixture containing Ferri et ammon. cit. gr. $1\frac{1}{2}$, aq. chlorof. ad m. 60 can be used. At first a few drops are given t.d.s. and the dose can be increased to m. 60 or 90 or

more t.d.s. as required. As an alternative measure a medicated dried milk can be used, containing ferri et ammon. cit. in such proportion that between 4 and 9 grains are given daily. It was found that in the infants treated with iron the morbidity rate was halved as compared with a control series not so treated, and the weight showed a definite increase in the treated cases.

Agranulocytic Angina and Malignant Neutropenia

The term " agranulocytic angina " was applied by Schultz (61) in 1922 to describe the illness of patients exhibiting severe progressive oral sepsis with marked prostration and neutropenia. Schilling (62) in 1929 suggested the alternative name of malignant neutropenia. Two varieties of acute cases are now described—primary agranulocytic angina and secondary malignant neutropenia. In the former, occasionally without any known cause, the patient is taken ill with fever, severe prostration and ulcerative lesions in the mouth or other mucous membranes, such as the vagina. There is a total reduction of the white cells, often to below 1,000 per c.mm. and a very low polymorphonuclear count. Many of these cases are now thought to be due to amidopyrine or to an amidopyrine and barbiturate compound, to which the patient has become susceptible by a preliminary sensitising dose.

Plum (63), in Copenhagen, recorded seven cases of agranulocytosis due to amidopyrine, and since 1933 many cases of agranulocytosis have been recorded developing after the therapeutic administration of amidopyrine preparations, such as Pyramidon, Gardan and Amidophen, or to barbiturates containing amidopyrine, such as Allonal, Cibalgin, Compral, Somnosal, Veramon and Veropyron. Agranulocytosis has also followed the administration of dinitrophenol, dinitrocresol, benzol, arsenic preparations, Sedormid, bismuth and gold salts. A fatal case following the administration of prontosil flavum has been recorded in Holland by Borst (64), and in America death has followed the use of sulfanilamide as recorded by Schwartz and his co-workers (65), and by Berg and his co-workers (66). It has also resulted from the use of other sulphonamide drugs, as described in Chapter I, especially if improperly given in small doses for prolonged periods, or in amounts totalling more than 25 to 30 g., and from the use of thiouracil, see Chapter XI. Both types of

disease, if untreated, in the majority of cases pursue a rapidly fatal course. The blood changes in malignant neutropenia resemble those of agranulocytic angina, but they are secondary to some infection such as pneumonia, sinusitis, osteomyelitis, staphylococcal septicæmia or liver abscess. Further, chronic cases of neutropenia have been described by Doan (67), in which the white cells from time to time fall below 4,000 per c.mm., owing to a reduction of the granulocytes, with accompanying symptoms of ill-health and a liability to septic infections. Barsby and Close (68) describe a case of recurrent agranulocytosis in which the symptoms recurred for three to seven days after the menstrual periods for four years, and during the last seven months of the patient's life, were accompanied by a neutrophil agranulocytosis.

Treatment. In 1924, Jackson (69) demonstrated the existence in normal human blood of pentose nucleotides. Jackson and his co-workers (70) in 1931 and 1932 reported the effect of treatment of cases of agranulocytic angina and malignant neutropenia with a preparation called "nucleotide K.96." This is now available as "pentnucleotide" and is put up in ampoules of 10 ml. containing 0·7 g.

In very acute cases 0·7 g. of pentnucleotide in 100 ml. of saline is injected slowly intravenously every morning for four days, and 0·7 g. of pentnucleotide in 10 ml. of distilled water is injected in the evening intramuscularly. The intramuscular injections are continued subsequently twice daily until the white cell count has definitely risen, and then an injection is given once a day until the white count has been essentially normal for three consecutive days. If there is myocardial degeneration the intravenous injection should not be given, owing to risk of heart failure from the reaction of dyspnœa and palpitations which is often provoked. In the average case only the daily intramuscular injections are given. The patient may appear worse during the first days of the treatment, but the improvement is usually definitely noted by the fourth or fifth day and the blood count returns to normal by the eighth or tenth day. In addition to the injections a careful search should be made for ulceration of the mucous membranes, or local abscesses elsewhere, and these should be appropriately treated. Blood transfusions are of doubtful value, but if the

patient is intolerant of pentnucleotide, as shown by symptoms of cardiac distress after a trial dose, a small transfusion (500 ml.) should be given. The use of penicillin in the treatment of agranulocytosis is described in Chapter II.

Results. Jackson and his co-workers (71) have reported sixty-nine cases treated by pentnucleotide by themselves and other physicians, with a recovery rate of 74%. In cases treated previously by various other methods a cure was only effected in 25% of cases. Cases successfully treated in England by pentnucleotide have been recorded by Bulmer (72), Marriott (73), Wilkinson and Israëls (74), and by Smith (75), and we have also treated a severe case of malignant neutropenia with good results. Fairley and Scott (76) report a fatal case of agranulocytic angina, in which there was a complete absence of response to nucleotide K-96 injections. It must, however, be remembered that the American observers report a recovery rate of only 74%. Israëls and Wilkinson (77) say that pentnucleotide has little effect in chronic neutropenia. For the latter Gupta and Witts (78) recommend the intramuscular injection of liver. It should be noted that pentnucleotide is useless in the treatment of aplastic anæmia, of leukæmia or of sepsis without neutropenia and leucopenia. We have also successfully treated with penicillin a case of agranulocytosis due to thiouracil.

Sternal Puncture

Ghedini (79) of Genoa, in 1908, introduced the method of human bone marrow biopsy by puncturing the shaft of the tibia and withdrawing a small amount of marrow for microscopical examination. Seyfarth (80), in 1923, recommended that the sternum should be trephined rather than the tibia, and Arinkin (81) in 1927, advised that a thick needle should be used for sternal puncture. Peabody (82), in 1927, investigated bone marrow, obtained by trephine from the tibia, in cases of pernicious anæmia. Salah (83), in 1937, described the needle which now forms the model of that used for sternal puncture. He used "a lumbar puncture needle (made of hard steel) with its stillet (*sic*) cut to 3 cms. length, its point sharpened but made broad so that it will not break. A movable shield is made to fit round the needle with a screw to fix it at the required distance."

The figures for the normal range of marrow cells have been worked out by Nordenson (84), Young and Osgood (85), Vogel, Erf and Rosenthal (86) and by Hynes (87). In some cases, especially in aplastic anæmia and myelosclerosis, it is necessary to know the histological structure of bone marrow, which can be done by removing a disc of bone with a 1 cm. trephine.

Method. The Salah needle is sterilised in hot oil. The patient is given an injection of morphine, gr. $\frac{1}{4}$, or aspirin, gr. 10 and nepenthe, m. 15, by mouth, half an hour before the operation. The skin over the manubrium sterni is sterilised with alcohol or iodine. The skin, subcutaneous tissues and periosteum are infiltrated with about 1 ml. of 2% Novocain or procaine solution, in the mid-line at the level of the third costal cartilage, using a fine intradermic needle with a short bevel. The patient lies on his back with a pillow between the shoulders. The Salah needle is then passed through the skin down to the sternum. The adjustable stop is fixed $\frac{1}{8}$ inch above the skin, and the needle is forced through the outer table of the bone by means of taps with a small hammer. When it is felt to enter the medullary cavity the stylet is withdrawn and a dry 1 ml. syringe is attached. Marrow juice is then sucked into the syringe. This produces a sensation of discomfort or even of actual pain. Hynes (87) recommends that 0·25 ml. of marrow fluid be withdrawn and placed in a tube containing Wintrobe's dry oxalate mixture. The tube is prepared by placing

TABLE I

Cells.	Neutrophil per cent.	Eosinophil per cent.	Basophil per cent.
Segmented polymorphs	9 –30	0–3	0–0·5
Non-segmented polymorphs	20 –40	0–2	0–1
Metamyelocytes	2·5–12	0–2·5	—
Myelocytes	2 – 8	0–1	—
Premyelocytes	0·5– 5		
Myeloblasts	0 – 2·5		
Lymphocytes	5 –20	Total cell count 25,000 to 100,000 per c.mm.	
Plasma cells	0 – 1		
Monocytes	0 – 5		
Normoblasts	7 –19	Myeloid-erythroblastic ratio 2 : 1 to 8 : 1.	
Erythroblasts	2 – 7		
Megaloblasts	0 – 4		

in it 0·1 ml. of a solution of 0·2% potassium oxalate and 0·3% ammonium oxalate and drying it in the incubator. Smears are then prepared, stained by Leishman's method, and a differential count is made of four or five hundred cells.

Normal Marrow Findings

The normal ranges in the bone marrow differential count, as described by Hynes (87), are shown in Table I. The cells in the lymphocyte series include the lymphoblast, when it is possible to distinguish it from the myeloblast, and a variety of intermediate forms. The first six types of cell in the table are myeloid in nature and the last three are erythroblastic.

Abnormal Marrow Findings

Leukæmia. Sternal puncture is of great value in the diagnosis of aleukæmic (leucopenic) leukæmia. The marrow changes here are similar to those found in leukæmia with leucocytosis.

The Acute Leukæmias. The marrow is very cellular, 70 to 99% of the marrow cells being primitive white cells either of the myeloid or lymphatic series. The proportion of primitive red cells is low, but the erythroblasts almost equal in number the normoblasts. There is no premature hæmoglobinisation of the erythroblasts, such as occurs in the bone marrow in pernicious anæmia. This maturation defect is probably associated with the macrocytic anæmia which is so often present in leukæmia.

Chronic Lymphatic Leukæmia. Here 40 to 90% of the marrow cells are lymphocytes, which may be normal small lymphocytes or show abnormalities in the size and structure of the nucleus. The myeloid cells and the erythroblasts show a maturation defect.

Chronic Myeloid Leukæmia. The marrow in early cases shows an increase in myeloblasts, premyelocytes and myelocytes. Lymphocytes and monocytes are few or absent. There is a maturation defect in the nucleated red cells. In the final stages of the disease the marrow resembles that found in acute myeloid leukæmia.

Pernicious Anæmia. In untreated cases 25 to 45% of the marrow cells are megaloblasts and erythroblasts, and premature hæmoglobinisation is present. The polymorphs are greatly decreased, but the number of earlier myeloid cells is not affected.

Aplastic Anæmia. The bone marrow should be studied by histological section and not by needle puncture. The marrow is usually hypoplastic, showing a lack of mature cells, particularly of the myeloid type, and an increase of primitive cells resembling small lymphocytes. In other cases the marrow is hyperplastic with many primitive cells. Israëls and Wilkinson (88) state that megaloblasts are not found.

Achrestic Anæmia. Israëls and Wilkinson (88) found megaloblastic hyperplasia resembling that of pernicious anæmia. Hynes (87) examined the marrow in one case and recorded an increase in the early hæmoglobinised erythroblasts and also in the normoblasts. The megaloblasts were normal. Mahler and Greenberg (89) found increased megaloblastic activity with diminished numbers of normoblasts.

Other Macrocytic Anæmias. In sprue and in carcinoma of the stomach marrow changes are found similar to those in pernicious anæmia.

Secondary Anæmias. Young and Osgood (85) demonstrated an erythroblastic reaction in the bone marrow in the anæmia which accompanies hæmorrhage, toxæmias and infections.

Polycythæmia. In some cases there is an increase in the erythroblasts and myeloid cells in the marrow. In others the only change found is an increase in the segmented polymorphs.

Acholuric Jaundice. In chronic cases the marrow is usually normal; in the acute phases, however, there is an increase of the normoblasts, erythroblasts and megaloblasts.

Agranulocytosis. In some cases there appears to be a maturation defect, the bone marrow having its normal number of cells, but all the myeloid cells being myeloblasts. In others there is an aplasia of the marrow, lymphocytes and plasma cells being the only white cells present.

Gaucher's Disease. The typical Gaucher's cells are present in the marrow.

Kala-azar. The Leishman-Donovan bodies are seen in the monocytes in the marrow.

Malaria. Parasites are more numerous than in the blood, being seen in the red cells and leucocytes in the marrow.

Myelomatosis. An excess of plasma cells may be present in the marrow.

Carcinomatosis. In secondary carcinoma of bone, carcinoma cells, in groups or isolated, are found in some instances in the marrow.

The appearances of the bone marrow in the abnormal conditions described above are largely based on the article by Hynes (87).

Conclusions. Sternal puncture is chiefly of value in the diagnosis of leukæmia with leucopenia, enabling a confident opinion to be expressed which cannot be done by blood examinations alone. It is also of value in the diagnosis of obscure cases of anæmia and certain other diseases, as described above.

Bone Marrow Transfusion

In some cases it is extremely difficult, if not impossible, to introduce fluids into the body by the direct intravenous route. This is likely to be so in adults if the veins are collapsed owing to shock, or if they are compressed by œdema of the subcutaneous tissues. Similar difficulties are met with in young children profoundly dehydrated as the result of gastro-enteritis, and in infants whose veins are either too small to receive the cannula or too tender to retain it even if successfully introduced.

That fluid may pass from the bone marrow cavity through the emissary veins into the general circulation was demonstrated by Tocantins and O'Neill (90) in America in 1936. Experimenting on the transplantation of marrow from animal to animal, they observed that if two needles were inserted into the marrow cavity of the rabbit's femur, one at either end of the bone, and 5 ml. of saline injected through the proximal needle, only about 2 ml. of fluid would come up through the needle at the distal end. Further investigations proved that the fluid passed direct from the bone marrow into the general circulation. Tocantins and O'Neill were not slow in applying their experimental results to clinical medicine, and, using the bone marrow of the sternum, the lower end of the femur and the upper end of the tibia, successfully infused citrated blood, citrated plasma, 5% dextrose solution and physiological saline into the general circulation both of adults and of children. These workers considered that the method was likely to prove of value in patients suffering from extensive burns, mutilating wounds, generalised œdema, circulatory collapse or where the veins are poorly developed or have been rendered useless by

repeated punctures or by injections of hypertonic saline. A case of diabetic coma was successfully treated by infusion of insulin and dextrose solution into the bone marrow, attempts at intravenous injection having failed, as the patient was pulseless and the veins were collapsed.

Hamilton Bailey (91) in 1944 reported on his experiences of the method in England and concluded that not only is the medullary cavity of the sternum as good a receptor as a vein for all kinds of infusions and for pentothal, to induce anæsthesia, but it is particularly valuable for the resuscitation of the very shocked and the very young. Again in 1946 (92) he recorded how a patient was rapidly revived after a severe surgical operation by the injection of over a pint of saline into the sternal marrow cavity, the infusion being completed within three minutes using a sternal puncture trocar and cannula and Record syringes.

Sternal Transfusion. Strict aseptic precautions must be taken to prevent the likelihood of osteomyelitis developing as a complication. In the method described by Hamilton Bailey (91) the patient lies flat and 2 ml. of 1% Novocain are infiltrated into the skin, subcutaneous tissues and periosteum in the midline just above the manubrio-gladiolar junction, the site of the injection then being massaged with a sterile swab so that the bony landmark can be clearly felt. The special trocar and cannula for sternal puncture is now pushed with a boring movement through the outer plate of the bone just above the manubrio-gladiolar junction, and directly the outer plate is felt to be penetrated the direction of the instrument is changed, the point being directed upwards towards the patient's head and the wings now prevent the point from piercing the inner plate of the bone. The trocar is withdrawn, and a Record syringe, half-filled with 3·8% sodium citrate solution, is attached to the cannula, a little citrate solution injected into the marrow cavity, and, on slowly withdrawing the piston, the syringe is seen to fill with the red bone marrow. Some more citrate is now injected, the syringe disconnected, and the cannula joined to the transfusion apparatus, the tubing of which must be free from air bubbles. The wings of the cannula, and the tubing are held in position by strapping attached to the chest wall, and the transfusion allowed to proceed. The container holding the fluid should be between 4 and 6 feet

above the level of the sternum ; if the pressure is too high pain will ensue. The flow of blood can be aided by oxygen pressure or by the use of a blood-saline mixture.

Tibial Transfusion. This is the method of choice for infants and young children. The leg must be maintained in a semi-externally rotated position by means of a splint, the other leg being loosely tied to the side of the cot to prevent dislocation of the cannula by kicking. Gunz and Dean (93) found that the legs were most satisfactorily controlled by firmly bandaging the soles of the feet to a padded splint, the feet being about 9 inches apart and the ends of the splint tied to the sides of the cot. Gimson (94) has devised four needles and stylets of different length and size for tibial transfusion in infants and young children (see Fig. 38). The flange is fixed and each needle has its own stylet. The needles measure below the flange, $\frac{1}{4}$ inch, $\frac{3}{8}$ inch, $\frac{1}{2}$ inch and $\frac{5}{8}$ inch. The two shorter needles are size 18 S.W.G., the two longer ones 16 S.W.G. The $\frac{1}{4}$ inch needle is for premature infants and the newborn, the $\frac{1}{2}$ inch and $\frac{5}{8}$ inch needles for children of about 5 years, but the size of the child and the amount of subcutaneous fat must be taken into consideration in selecting the needle. The handle (see Fig. 38) is screwed on to the stylet, and the needle and stylet inserted, after preliminary cleaning of the skin and anæsthetisation with Novocain, into the subcutaneous surface of the tibia, and at right-angles to the subcutaneous bony

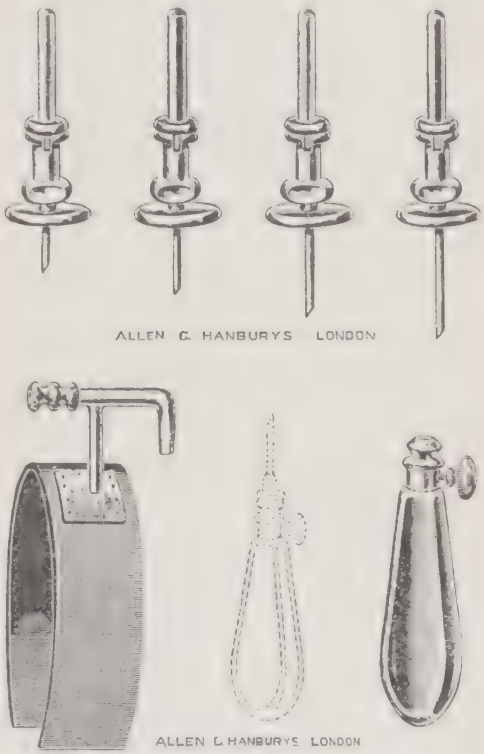

FIG. 38. Needles etc., for bone-marrow transfusion.

plate. The site selected is inferior and medial to the anterior tibial tuberosity, below the epiphyseal line and above the nutrient artery. The flange rests on the skin, the point of the needle being in the marrow cavity. The stylet is withdrawn and a small amount of marrow wells up or is sucked up into a syringe partly filled with saline attached to the needle. A little saline is injected into the marrow cavity to make sure that the needle is in position. The adaptor (see Fig. 38), filled with transfusion fluid and connected to the tubing from the dripper and bottle from which air bubbles have been excluded, is fitted to the needle and the transfusion begun. A screw clip on the tubing can be used to adjust the rate of flow, and in one case Gimson maintained the transfusion for as long as six days. Owing to the difficulty in judging the size of needle required, Behr (95) prefers for tibial transfusion a modification of the Hamilton Bailey trocar and cannula, furnished with adjustable wings. Hartmann's solution is valuable as a transfusion for infants suffering from dehydration due to gastro-enteritis, as it maintains the acid-base equilibrium. It contains sodium chloride 6 g., potassium chloride 0·4 g., calcium chloride 0·2 g., lactic acid 2·4 ml., N/10 NaOH q.s. to neutralise the lactic acid, and water to 1,000 ml. There may be difficulty in transfusing blood, if undiluted, even if the container is raised to as much as 7 feet above the needle, and Gunz and Dean (93) considered the method unsuitable for the transfusion of blood. For dehydrated infants Behr (95) recommends the transfusion of half strength Hartmann's solution in 5% dextrose, the rate of flow being varied according to the weight of the patient. For infants weighing less than 5 lbs. the flow should be 4 drops a minute, if under 7½ lbs. 6 drops a minute, under 10 lbs. 8 drops a minute, and under 15 lbs. 12 drops a minute. The drops apply to a drip-bulb delivering 1 ml. in 16 drops.

Heparin

McLean (96), in 1916, extracted from dog liver a substance which retards the coagulation of blood *in vitro*. Further investigations were carried out by Howell and Holt (97) in 1918 and the anti-coagulant substance was called heparin. In 1923 Howell and Holt (98) described an improved method for its preparation and defined the unit of heparin as being that weight of substance which

would prevent 1 ml. of cat blood from clotting for twenty-four hours when kept in the cold. McHenry and Glaister, at Best's suggestion, subsequently prepared small quantities of heparin from beef liver, and Charles and Scott (99), in 1933, evolved a process by which it could be prepared in much larger quantities from liver. They also obtained a blood anti-coagulant, presumably heparin, from skeletal muscle and lung. In 1936 the same workers (100) prepared a crystalline barium salt of heparin having a high potency of about 500 units per mg. They gave the empirical formula of heparin as $C_{25}H_{65}O_{50}N_2S_5$. By treating the crystalline barium salt with excess of ammonium carbonate solution at 65° C. the barium was removed, the heparin was then precipitated by acetic acid, centrifuged down, washed with ether and dried. For use a solution is prepared to which tricresol is added, and it is sterilised by passing through a Berkefeld filter. Jorpes and Bergstrom (101) classified heparin as a mucoitin polysulphuric acid, closely related to chondroitin-sulphuric acid. Best (102) has suggested as a standard that the unit is the activity contained in 0·01 mg. (10γ) of the barium-free material, *i.e.*, 100 units in 1 mg. There is evidence to suggest that heparin is either formed in, or stored in the mast cells, as had been shown by Jorpes, Holmgren and Wilander (103).

Mode of Action. It is believed by Howell that heparin is normally present in the blood. Howell and Holt (97) showed that heparin has no effect in preventing coagulation when added to mixtures of thrombin and fibrinogen, and differs in this respect from hirudin. They suggested that it delays coagulation by inhibiting the activation of prothrombin to thrombin. Mellanby (104) considered that heparin acts as an antithrombase and says it is improbable that heparin is responsible for the normal fluidity of blood. He suggests that heparin is concerned with the prevention of localised thrombosis in the immediate neighbourhood of disintegrating tissue. Best (105), in 1939, upheld the view that heparin apparently combines with the antithrombin of the serum albumin fraction and thus acts as an anti-coagulant by increasing the affinity of this substance for thrombin.

Clinical Applications. Heparin has been used in surgery, in medicine and in blood examinations. Heparin is available in sterile solution in rubber-capped vials of 3 ml. ($\frac{1}{2}\%$ = 5 mg. per

ml.); of 5 ml. (1% = 10 mg. per ml.), and of 5 ml. (5% = 50 mg. per ml.). This preparation contains 500 units per mg. Heparinised tubes (10 ml.), each containing 1 mg., are available for collection of blood samples. In man 1 mg. of heparin per kg. body weight, when given intravenously, prolongs the coagulation time to approximately forty minutes. For clinical use the average dose for an adult is 75 to 150 mg., given intravenously in the form of a 5% solution. This dose is repeated every third or fourth hour, four or five times a day, and the effect should be checked by determination of the coagulation rate, which should be kept at about fifteen to twenty minutes by the Lee and White method (normal four to seven minutes).

Surgical. Murray et al. (106), after showing that heparin will prevent thrombosis resulting from mechanical and chemical injury to blood vessels in dogs, first administered heparin to a patient in a surgical ward of the Toronto General Hospital in 1935. The solution was injected into the brachial artery under local anæsthesia, and samples of blood from the veins of the injected arm showed a rise in clotting time from the normal of six minutes to that of eighteen minutes. Subsequently heparin was administered post-operatively as a prophylactic measure against thrombosis. The injections were commenced two to three hours after the operation and were continued for twenty-four to 120 hours. An effort was made to keep the clotting time at fifteen to twenty minutes. The operations included appendicectomy, herniotomy, resection of colon, etc. Heparin has also been used in arteriotomies for embolism. Crafoord (107) advises that in these cases it may be necessary to start heparinisation during the operation. When heparin has thus been given, or when hæmorrhage unexpectedly sets in during the course of heparin treatment, the original coagulation time of the blood may be restored by the injection of protamine. Chargaff and Olson (108) described this neutralising effect on heparin, and Jorpes et al. (109) state that 60 mg. of protamine (Clupein) instantaneously neutralise the effect of 100 mg. of heparin. They say, however, that the protamine injection should only be used as a last resort, and a blood transfusion will usually effect the necessary hæmostasis. Richmond (110) records a case which shows that the intravenous injection of heparin to prevent post-operative thrombosis is not devoid of

danger. An intravenous injection of 20 mg. of heparin in normal saline was given to a girl aged eleven years after removal of a twisted ovarian dermoid. Massive hæmaturia occurred sixteen hours later, the blood coagulation time being seventeen minutes. The patient was very collapsed, but recovered with bladder irrigations and a blood transfusion.

Medical. Ploman (111) used heparin in the treatment of acute thrombosis of the central vein of the retina. Intravenous injections of a 5% heparin solution were given with a fine needle. Four injections were made daily, the first three of 50 mg. and the last of 100 mg. Marked improvement or full restoration of vision was obtained. Magnusson (112) recorded a case of thrombosis of the posterior inferior cerebellar artery in a woman aged forty-eight. Intravenous injections of a 5% solution were used; 150 mg. in the morning and afternoon of the first day, and the same amount three times a day for five days. He states that the case was a severe one and that improvement was more rapid than would be expected normally. Kelson and White (113) have reported some favourable results in the treatment of subacute bacterial endocarditis by the combined use of heparin and sulphapyridine. Their method is as follows: The contents of a 10 ml. vial of heparin (10,000 units) are added to 500 ml. of normal saline and the solution is given by continuous intravenous drip for fourteen days. The rate of flow, usually 15 to 25 drops per minute, is regulated to maintain as well as possible the venous clotting time at approximately one hour. Sulphapyridine, g. 4–6 daily, is given by mouth for a week before, during and a week after the heparin treatment. Blood transfusions are given if there is an anæmia of 3·5 million red cells or lower. All patients are saturated with ascorbic acid, 200 mg. being given by mouth four times a day for three days and subsequently 100 mg. a day.

We have used this method in 2 cases of subacute bacterial (streptococcus viridans) endocarditis without benefit, the patients dying a few weeks later. If the sulphapyridine, which is given before the heparin, fails to sterilise the blood, there appears to be an increased risk of the occurrence of vascular accidents, such as cerebral hæmorrhage. McLean, Meyer and Griffith (114), reviewing 67 cases treated by this method, report very unfavourably on it, and it appears probable that it will be abandoned.

Heparin has also been used in the treatment of thrombophlebitis, including the migratory type, in pulmonary embolism and in coronary thrombosis. Murray (115) states that in phlebitis the effect of heparin is most noticeable when given early in the disease. In the migratory type, although the heparin will probably relieve the symptoms of thrombosis, it will not affect the cause of the disease. In an average case of thrombophlebitis, heparin is given for ten days. After the first three to four days the patient is encouraged to move actively in bed, and he should get up by the tenth to twelfth day. Murray (115) records that 22 cases of massive pulmonary embolism have been treated with heparin with pleasing results; no patient died from the embolism, and dyspnœa and distress were rapidly relieved. Only a few cases of coronary thrombosis have been treated, but experimental results indicate that heparin may prevent the occurrence of mural thrombosis with subsequent risk of peripheral embolus formation. Stansfield (116) treated successfully a case of puerperal cerebral thrombophlebitis with heparin, using an intravenous drip of 5% dextrose saline, containing 200 mg. heparin in each pint. The coagulation time was prolonged to forty minutes by this treatment. Other cases of recovery have, however, been recorded without the use of heparin. Massive hæmaturia has resulted from the treatment of a case of cavernous sinus thrombosis with sulphathiazole and heparin, as described by Ershler and Blaisdell (117). The evidence given in the article indicates that the bleeding was due to the heparin and not to the sulphathiazole.

Blood Transfusions. Hedenius (118), in 1937, reported on the use of heparin in blood transfusion. A 5% solution is used, 1 mg. per kg. body weight being injected intravenously into the donor. The blood coagulation time is prolonged rapidly at first and returns to normal in one and half hours. Ten minutes after the injection blood is removed from the donor with any standard equipment, and the whole blood in its natural state injected into the recipient. If syringes are used the plunger should be moistened with a drop of sterile paraffin to prevent cell destruction and kinase production. The coagulation rate of the recipient is not affected. The technique employed in citrate transfusions may also be used, heparin being added to the drawn blood instead of citrate, using 20 mg. heparin (0·4 ml. of a 5% solution diluted with

10 ml. sterile saline) for 500 ml. of blood. These methods have also been reported on favourably by Sappington (119) in America.

Blood Examinations. Heparinised blood may be used for biochemical and cytological examinations, but is not suitable for performing the Wassermann reaction. The blood is placed in a 10 ml. tube containing 1 mg. of heparin, which is distributed as a smear on the inside of the tube. A leucocyte count should be carried out within two hours of collecting the blood.

Conclusions. It is evident that the administration of heparin is not devoid of danger from cerebral or renal hæmorrhage, and its use seems only to be justified in cases of such gravity that death or permanent disability appears probable if heparin is withheld.

Plasma Transfusion

The special problems of war have led to great advances in the technique and knowledge of blood transfusion, and the value of stored blood is now established. In many cases, however, where transfusion is needed it is the fluid element and not the red cells which is the important factor, and in wound shock, post-operative shock and burns, plasma is being increasingly used. Brennan (120) drew attention to the advantage of plasma transfusion in the treatment of hæmorrhage.

The practical advantages of plasma over stored blood are plain ; blood is useless after being kept from fourteen to twenty-eight days and correct grouping is essential. Plasma is obtained by the simple process of standing blood for twenty-four hours, or by centrifuging, and can either be kept in a sterile bottle with an equal quantity of saline or evaporated *in vacuo* to a dry powder and reconstituted by the addition of distilled water. It can be stored for an indefinite period without elaborate refrigeration, and no grouping is necessary. The latter means great saving of time, and plasma transfusions can often be given within five minutes of the patient's admission.

The Blood Groups

The realisation that the blood of human beings may be divided into four main groups is due to the work of Landsteiner (121),

Jansky (122) and Moss (123). These groups result from the presence or absence of two antigens or agglutinogens A and B in the red cells and two antibodies or agglutinins α (anti-A) and β (anti-B) in the serum. Hæmagglutination occurs when red cells containing the agglutinogen A are injected into a person whose serum contains the agglutinin α, or when red cells containing the agglutinogen B are injected into a person whose serum contains the agglutinin β. The agglutination is followed by hæmolysis of the injected cells and may lead to the death of the patient. In this country the classification of Moss or of Landsteiner is usually adopted; both of these classifications are given in the accompanying table :—

Landsteiner	Moss	Agglutinogen in cells	Agglutinin in serum
A B	1	A B	O
A	2	A	β
B	3	B	α
O	4	O	$\alpha + \beta$

The guiding rule for transfusion is that the donor's cells should not be agglutinated by the recipient's serum. A patient belonging to group A B (1) is a universal recipient and may receive cells from any group, a group A (2) patient may receive blood from groups A (2) and 0 (4), a group B (3) patient may receive blood from groups B (3) and O (4), and a group O (4) patient may receive blood from group O (4) only.

At birth the agglutinogens are detectable, but agglutinins are present in only half of newly born babies, and are identical with those of the mother. Smith (124) found that these agglutinins diminish in titre during the first ten days of life and are replaced by agglutinins formed in the body of the infant. The groups become established at about one year and the titres of both agglutinogens and agglutinins gradually increase, reaching adult strength at about the age of twenty. After the age of forty the titre declines. It is generally believed that once a person's blood group is established a change to another group is impossible. The distribution of the groups varies with the race and geographical position. The following distribution is given by Taylor, Race,

Prior and Ikin (125) as representative of the United Kingdom: Group A B, 3·2%; group A, 42·2%; group B, 8·7%; group O, 45·8%.

Sub-groups. Group A is subdivided into A_1 and A_2, and Group A B into A_1B and A_2B. The difference between the agglutinogens A_1 and A_2 is both qualitative and quantitative. The qualitative difference is shown by the occurrence in plasma of two agglutinins α_1 and α_2, although these are rarely found. The quantitative difference is demonstrated by the relative insensitivity of the A_2 factor in reacting with agglutinin α. This behaviour is important since, unless very potent anti-A serum is employed in testing for A_2, this factor is liable to be missed, and blood belonging to group A_2 may erroneously be classed as group O, while blood of group A_2B may be returned as belonging to group B. Group A_1 is encountered about four times as frequently as group A_2, while group A_1B is one and a half times as common as A_2B. The occurrence of agglutinogens and agglutinins in these groups is given in the following table :—

Group	Agglutinogen in cells	Agglutinin in serum
A_1	A_1	β (rarely $\beta + \alpha_2$)
A_2	A_2	β (rarely $\beta + \alpha_1$)
A_1B	A_1B	O (rarely α_2)
A_2B	A_2B	O (rarely α_1)

In 819 individuals tested by Wiener (126) no α_2 agglutinin was found in groups A_1B or A_1, but about one in 200 contained α_1 agglutinin in groups A_2 and A_2B. It has been shown that α_2 is also able to agglutinate cells of group O, and, for this reason, this factor, although rare, is a greater danger than α_1. In general, the transfusion of group A_1 blood to a person of group A_2, and *vice versa*, is without danger, especially for a first transfusion, but during pregnancy and the puerperium, special care is necessary since the iso-immunisation of an A_2 mother by an A_1 fœtus was encountered by Wiener (126). In the rare event of α_1 or α_2 being present in quantity, however, a reaction may occur, or the cells may undergo slow hæmolysis. The main danger lies in the increased production of antibody, with the result that a second

or subsequent transfusion with the same blood is liable to provoke a severe reaction.

The Diagnosis of the A B O Blood Groups. The correct diagnosis of the A B O blood groups is a matter of vital importance. Nevertheless, errors may sometimes occur if the indirect test, which consists of testing the red blood cells for agglutinogens, is the sole test employed. Among the errors which may lead to a wrong diagnosis, Taylor, Race, Prior and Ikin (125) list the following, while putting forward a technique which is thought to give completely accurate results : 1. Clerical errors, including the incorrect recording of results. 2. Technical errors due to (*a*) using wrong reagents ; (*b*) false positive reactions resulting from inexperience, cold agglutinations, pseudo-agglutination or faults in the red cell suspension, *e.g.*, infection ; (*c*) false negative reactions due to the use of weak grouping sera ; the weaker A_2 factor in the subgroup A_2B is commonly missed and the cells are diagnosed as belonging to group B.

Some of the advantages of the new technique are (1) the cells are examined for agglutinogens, and, later the agglutinin content of the serum is determined ; (2) the tests are made in tubes ; (3) no reaction is recorded as negative without a microscopical examination.

Apparatus. Fifty-hole wooden blocks, holding five rows of 10 tubes, are used as holders. In this way, without using the centre row of holes, twenty samples may be examined for red cell antigens or serum antibodies simultaneously. The tubes are 2 in. by $\frac{1}{4}$ in., without lip, and are covered with caps consisting of flat-bottomed glass tubes 25 mm. by 11 mm. The caps are put on the tubes after the addition of the reagents and serve to exclude dust and minimise evaporation, as well as acting as place-markers when they are removed prior to the reading of the reactions in the tubes. Pasteur pipettes fitted with teats, and marked to deliver 0·08 and 0·04 ml., and 0·85% sodium chloride in distilled water, are also required.

Method. Red cell suspensions should be obtained by bleeding into 3% sodium citrate. Saline is used to dilute the suspension to about 5% of whole blood, as judged by eye. In other cases cells are recovered from the blood clot, washed with saline and resuspended in the same liquid. The grouping serum is stored in

small containers and is frozen solid. Each time it is used it is thawed and refrozen as soon as possible. Anti-A (α) serum is diluted with an equal volume of saline, and 0·08 ml. of the mixture is placed in the tubes of the first and fourth 10-hole rows. A similar quantity of anti-B (β) serum, also diluted with an equal quantity of saline, is placed in the tubes of the second and fifth rows. The dilution with saline is necessary to prevent pseudo-agglutination. 0·04 ml. of the red cell suspension from the first sample of blood is placed in the first tubes in the first and second 10-hole rows and the cells from the second sample in the second tubes of the same rows, etc. As controls, cells of known blood groups O, A and B are included in each batch of tests, as it is essential to be certain that the sera are reacting with appropriate cells and only with appropriate cells. After receiving the reagents, the tubes are shaken sufficiently to yield even suspensions of cells, covered with caps, and left at room temperature for two hours. At the end of this period each tube is picked up, uncovered, and tapped sharply three or four times with the finger. Clumping is sought by holding the tube to the light, and the contents of each tube not showing naked-eye agglutinates are examined with the microscope by spreading a small portion of the well-mixed contents evenly on a slide with the stem of the Pasteur pipette. The film is viewed without a cover-slip, under the low power of the microscope.

Tests for Agglutinins in Serum. When checking the antibody content of the serum, 0·06 ml. of each serum and 0·06 ml. of saline are placed in each tube of the pair of tubes allotted to each specimen, using a Pasteur pipette with only one marking. To the first tube of the pair is added 0·06 ml. of a suspension of known A cells, to the second 0·06 ml. of known B cells. The tubes are shaken, capped and read after two hours as described above.

System of Scoring the Strength of the Reaction. C (complete) = clump of cells in clear surrounding fluid visible to the naked eye. V (visual) = a somewhat weaker reaction with clumps distinctly visible to the naked eye, although the surrounding fluid is not clear. + + = very big clumps under the microscope. + = not quite such big clumps. (+) = less than +, clumps of 8–12 cells. W (weak) = a definite but weak reaction in which there is

a uniform distribution of very small clumps of about 4–8 cells. ? = uneven distribution of cells with no very definite clumps.

Further Tests. Some difficulty may at times be encountered in the diagnosis of the A factor in group A B, since the reactions of the agglutinogen A when present with B are often markedly weaker. This is especially the case with the weaker A_2 agglutinogen. The examination of the serum for antibodies does not necessarily show that such a case, which may be diagnosed as group B, is really A B, since the serum from a number of A_2B cases contains the antibody α_1, which reacts with A_1 but not with A_2 cells. If such serum is mixed with test cells of the type A_1, a reaction will result which seems to confirm the diagnosis of group B. Therefore, the serum of every blood sample which has been shown by the determination of its antigen and antibody content to belong to group B is tested with known A_2 cells. If there is a reaction the serum cannot be that of an A_2B person. Occasionally cell suspensions, which are not in good condition, give rise to false positive reactions. This is probably due to the cells being infected. In all anomalous cases, the tests are repeated with cells which have been recovered from the clot, washed, and resuspended in saline and with a fresh portion of serum. However, it is better to obtain a fresh specimen of blood. When, as sometimes happens, the serum of a case of group A or of group AB gives a reaction with known A_1 cells, the serum is tested with known A_2 cells. If no reaction occurs, it is assumed to contain α_1, which may be present in association with A_2 or A_2B agglutinogens. Very occasionally, a serum expected to react with B cells fails to do so, owing to a deficiency or absence of the anti-B agglutinin. If, after repetition, this observation is confirmed, diagnosis should be based on the reaction given by the red cells.

The Direct Test. In addition, the two bloods should always be cross-matched immediately prior to the transfusion in order to eliminate all chances of error. The technique of the test is described at the end of the following section on the Rh factor. If the result is doubtful the donor should be rejected.

The Rh Factor. The Rh factor is an antigen which may be present in human red cells and is independent of the ordinary Landsteiner groups. It was discovered by Landsteiner and Wiener (127) in human erythrocytes, which were found to react

with anti-rhesus sera prepared by injecting blood from rhesus monkeys into rabbits. Blood which reacts in this way is termed Rh-positive, while blood which is not agglutinated in this test is termed Rh-negative. In a further investigation Wiener (128) examined 777 persons and found that 14·4% of them were Rh-negative. This result was confirmed in this country by Boorman, Dodd and Mollison (129), who found that 14·85% of the 1,610 blood donors they examined were Rh-negative when tested with human anti-Rh sera. It is probable that racial differences in distribution of the Rh factor exist, since a much lower incidence of Rh-negative individuals has been reported among American Indians. According to Landsteiner and Wiener (130) the Rh factor is inherited as a simple Mendelian dominant.

The importance of the Rh factor in clinical medicine lies in the fact that individuals whose red cells are Rh-negative are capable, in certain circumstances, of forming an antibody which reacts with the Rh antigen if this is introduced into the body. This condition was first demonstrated by Wiener and Peters (131), who in 4 cases encountered hæmolytic reactions after the repeated transfusion of supposedly compatible blood. These authors showed that in 3 cases the patients were Rh-negative and that after transfusion with Rh-positive blood they developed an agglutinin in their sera which reacted in the same way as anti-rhesus serum. Later Levine, Katzin and Burnham (132) drew attention to the high incidence of erythroblastosis fœtalis in infants born of mothers whose blood contained atypical agglutinins which, in most cases, gave reactions identical with those of anti-Rh sera. Landsteiner and Wiener (130) attributed these findings to the possibility of an Rh-negative mother and an Rh-positive father giving rise to an Rh-positive child. The mother may then develop anti-Rh agglutinins to counteract the Rh agglutinogen in her fœtus. The passage of the anti-Rh agglutinin across the placenta to the fœtus is held to be responsible for the erythroblastosis. These observations were extended by Levine, Burnham, Katzin and Vogel (133) who found that of 153 mothers whose infants were affected with erythroblastosis 92% were Rh-negative, and of 76 infants and 89 husbands in this group who were examined, all were Rh-positive. In a later investigation, Boorman, Dodd and Mollison (129) examined 48 cases of erythroblastosis fœtalis and

found anti-Rh agglutinin in the serum of 44 mothers, and either anti-A or anti-B in the remaining cases. They concluded that anti-A and anti-B agglutinins were also capable of causing destruction of the infant's erythrocytes in a certain number of cases.

It would appear that the transfusion of Rh-positive blood to an Rh-negative individual is unlikely to lead to a severe reaction at the first transfusion, except during pregnancy and during the puerperium. However, Wiener (134) considers that many of the hæmolytic reactions which occur when homologous blood is transfused are due to the Rh factor, and that Rh-negative blood should be used for the repeated transfusion of Rh-negative individuals. Boorman, Dodd and Mollison (129) suggest the establishment of a panel of group O Rh-negative donors at every blood bank to supply the requirements of the cases mentioned above. These authors recommend the following technique for the detection of the Rh factor.

Testing Unknown Cells. A suspension of the cells to be tested is prepared by adding one small drop (0·02 ml.) of blood to 1 ml. of saline; the final concentration should be 1 to 2% in terms of blood sediment. One volume of this suspension is placed with 1 volume of the test serum in a small tube 7 mm. in diameter. It is desirable to test cells with not less than three different sera. At the same time, known Rh-positive and Rh-negative cells should be put up with the same sera to serve as controls. The tubes should be left for at least one hour, and preferably two hours, and kept at 37° C. whenever possible. The sediment is then examined macroscopically and microscopically. Usually, definite clumping is seen in blood taken from the tubes giving macroscopic positives. Occasionally, however, only very small agglutinates are seen despite a characteristically positive sediment, and harsh treatment of the mixtures in such cases sometimes completely abolishes the agglutination. It is better to rely on seeing agglutinates macroscopically or microscopically as a sign of a positive reaction, and to regard the sediment pattern simply as a guide to the reaction. The danger of false negatives is reduced by the use of three different sera. If sufficiently potent sera can be obtained, it is advisable to add a volume of saline to the volume of serum and of cells in order to diminish roulcaux formation.

Testing Unknown Sera. Sera suspected of containing Rh antibodies should be tested against at least 10 different group O bloods collected at random, and also with not less than two known Rh-positive and two known Rh-negative bloods, using the technique described above. If anti-Rh is present, the reactions should be positive with the known Rh-positive bloods, negative with the known negatives, and positive with the majority of the unknown group O bloods. To exclude the presence of powerful autoagglutinins the serum should be tested with the cells of the individual from whom it came.

Direct Matching Test. If the recipient's serum is considered to contain Rh agglutinins, a suspension of the donor's blood should be mixed with the recipient's serum in a small tube and allowed to stand for one to two hours, preferably in the incubator. The sediment is examined macroscopically and microscopically and the least evidence of agglutination must be taken as a definite contra-indication to the use of the particular donor.

When it is desirable to complete the test as soon as possible, the tube containing the mixture of cells and serum may be centrifuged slowly at the end of the thirty minutes at the rate of 500 r.p.m. for one minute. The tube is then gently shaken and the contents examined.

It should be pointed out that after the injection of Rh-positive blood it may not be possible to demonstrate the presence of Rh-antibodies in the recipient's serum. After a few days the strength of the antibody increases and reaches a maximum after one to three weeks. In the case of repeated transfusions, it is obviously essential to test the two bloods immediately prior to a transfusion.

Hazards of Blood Transfusion. These have been dealt with at length by Whitby (135) in a valuable review from which much of the following material has been drawn. The hazards are divided into four categories :—

1. The systemic non-specific pyrexial type of reaction.
2. Circulatory and vascular disasters due to the general condition of the patient.
3. Intravascular haemolysis of the transfused blood.
4. Disease transmitted from the donor.

Systemic Reactions. Riddell (136) has placed febrile reactions in three grades : (*a*) Those in which the temperature rises to 100° F.

without untoward symptoms. (*b*) Those showing a rise in temperature to 100° F. or above associated with a feeling of cold without rigor. (*c*) Those associated with rigor. Many of these reactions are avoidable and have been tracked down to insufficient attention to details of the technique or to personal factors such as the condition of the patient and the speed at which the operator carries out the transfusion. It has been shown by Maycock and Whitby (137) that too fast a rate of transfusion can cause a rigor, which ceases instantly when the rate is slowed. Pyrexial reactions are often caused by foreign protein such as dead bacteria or living non-pathogenic bacteria being introduced into the body. The use of freshly and properly distilled water for the preparation of solutions, etc., and the adoption of a scrupulous cleansing technique with regard to apparatus have been shown to reduce greatly the incidence of pyrexial reactions. It is also important when using stored blood to avoid delay after the blood has warmed up following removal from the refrigerator.

Circulatory Reactions. Wartime conditions frequently necessitate large transfusions. If transfusions of the order of 4 to 7 pints of blood are given, the possibility of the circulation becoming overloaded should not be overlooked. Recent work has indicated that the normal person can receive from 1 to 3 pints of serum in 7 to 20 minutes without the development of any undue symptoms, and Whitby (135) is of the opinion that failure to restore blood-volume in a casualty is a danger greater than overloading of the circulation, except in the presence of an enfeebled cardiac muscle. In such a case, the rate should not exceed 1 ml. per lb. of body weight per hour, and should be reduced to half this figure if warranted by the poor condition of the patient. The danger of overloading is greatly reduced by the use of concentrated red cell suspension transfusions.

Intravascular Hæmolysis. This is usually due to the use of incompatible red cells in the transfused blood caused by an error in grouping. Although the question of the compatibility of the two bloods has been discussed in the previous pages, a few additional points in connection with this important subject deserve attention. It is possible, with universal but not with homologous donors, for the transfused plasma to contain sufficient agglutinins to destroy the red cells of the recipient. This condition rarely

occurs because such high titre donors are uncommon and supplies of A and B agglutinogens are not confined to the recipient's red cells. During plasma transfusion, the danger from this source is reduced by bulk pooling of the plasma, whereby the potency may be reduced by dilution and by absorption by the small quantity of agglutinogen known to be present in plasma. Witebsky, Klendschoj and Swanson (138) have reported the preparation of pure A and B specific substances which are capable of absorbing the agglutinins of plasma, but, as yet, it has not been possible to apply this process on a large scale. A second hazard is the presence of well-developed cold agglutinins or auto-agglutinins in the recipient's blood, which may cause a reaction if the patient is transfused with cold blood. This factor is active in causing hæmagglutination only at 30° C. or below, and is present in paroxysmal hæmoglobinuria and other diseases. In such a case the blood must be transfused while warm. Difficulty is likely to be encountered in determining the blood group of such a patient since the indirect test indicates group A B, whereas the direct test suggests incompatibility even with group O blood. The correct group must be determined by repeating the test at 37° C. The risk of dangers from factors such as M, N and P appears to be negligible since no naturally occurring agglutinins to these factors have been reported. Stored blood is generally regarded as a satisfactory substitute for fresh blood if used within fourteen days, but after that period the number of transfused corpuscles which survive decreases, and degenerate stored blood is rapidly hæmolysed.

Transmissible Disease. Donors suffering from syphilis and malaria should be avoided since such diseases may be communicated to the recipient in the transfused blood. Allergic reactions, such as urticaria, which occur in 1% of cases, are probably due to the patient being sensitive to the transfused plasma proteins and are most commonly observed after repeated transfusions from the same donor. Another possibility is the presence of bacteria in stored blood, unfiltered plasma or serum. For this reason the refrigerated specimens need careful inspection and on no account should a specimen of blood taken from store be warmed up and replaced in store for use on some future occasion.

Conclusions. In general, it may be stated that little risk is attached to a first transfusion provided the bloods have been correctly grouped. However, during pregnancy and the puerperium special care is necessary even at a first transfusion, since additional factors, *e.g.*, the Rh factor, must be considered. Great care should be taken whenever multiple transfusions are given, since a Rh-negative individual may react if repeatedly transfused with Rh-positive blood from the same donor. As a final check, it is a good plan to note the effect of injecting 10 to 20 ml. of blood before giving the whole transfusion. In addition, it is preferable to transfuse homologous blood whenever possible, since the frequent use of universal donors, although often necessitated by circumstances, does not always yield such satisfactory results as blood from the same group.

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CHAPTER XIV

BLOOD AND URINE ANALYSIS

The essential requirements of methods used for routine analyses are accuracy, speed and ease of operation. The fact that colorimetric methods possess all these qualities has inevitably led to their widespread adoption in the clinical laboratory. Volumetric methods lack nothing in accuracy and are still employed, but are set at a disadvantage because in general they require a large volume of blood and involve somewhat more difficult and lengthy procedures.

The emphasis which formerly lay on the direct vision colorimeter has moved somewhat in favour of photo-electric comparison. The following accounts apply to two types of instruments which are now in general use.

The Direct Vision Colorimeter

The optical arrangement of this type of colorimeter can be seen in the diagrammatic sketch (see Fig. 39). In practice the colour intensity of the unknown solution is matched by visual judgment with that of a standard coloured solution.

The solutions compared, standard and unknown, are contained respectively in two colorimeter cups, the vertical walls of which are opaque, whilst the bases are transparent. These cups are mounted on platforms which can be moved vertically. A transparent plunger dips into each cup and the effect of racking the cup platform up and down is to vary the depth of solution viewed by the eyepiece.

This eyepiece, which is part of the optical system, gives a coloured image of the contents of the cups. The light, either daylight or from a lamp source, preferably mounted in the base of the instrument, is incident upon the bottom of the cups and passes by the paths shown through solutions, plungers and prism. The emergent light is focussed by the optical system so

that, viewed from the eyepiece, the image consists of a circular field divided diametrically into two, each semicircle corresponding to one of the two solutions examined.

Before use the zero adjustments of the instrument should be checked. It is essential, in the first place, that when the plungers are touching the base of the cups the vernier scales should be at zero. In modern instruments it is possible to adjust the zero, but failing this the error must always be compensated. In the second place, before making a reading, the illuminating source must be centralised. Both cups are filled with standard solution, the vernier scales are set at the same level, usually 20 mm., and the light is then adjusted until the field is uniform.

The standard solution in the right cup is then replaced by the unknown solution and the colours are again matched by varying the depth of the unknown solution.

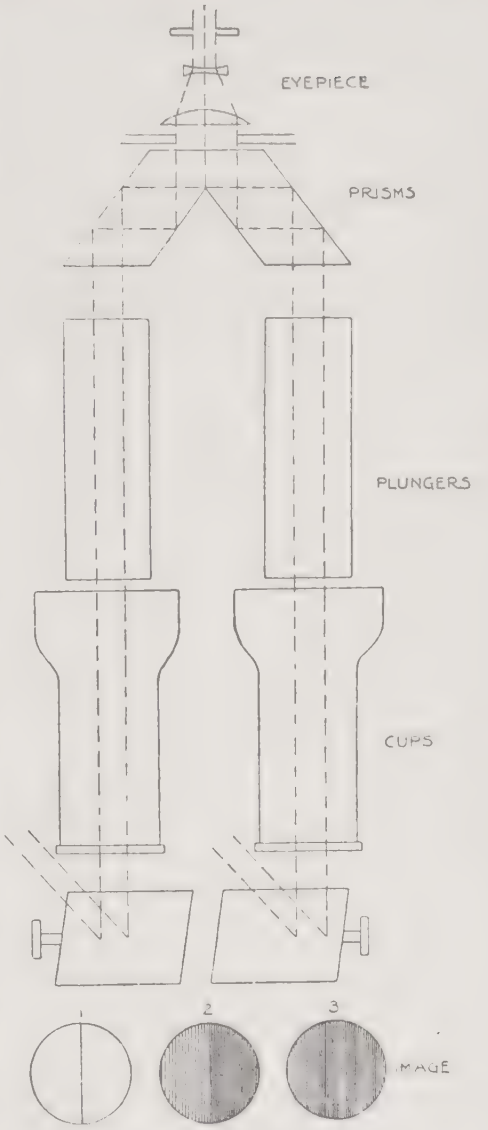

FIG. 39. The Colorimeter.

1. Represents the appearance when the cups are empty.
2. When too great a depth is observed in the right cup.
3. Represents the match.

It is in this final colour matching that a personal error is introduced, which limits the accuracy of this type of colorimeter. It

is recommended that a balance be struck as a result of a series of glances of short duration, rather than by a prolonged stare, for fatigue of the retina leads to inaccurate matching. The use of selective light filters will be found of great advantage in this connection, particularly when matching colours in those regions of the spectrum where the eye is relatively insensitive. As far as possible filters should be selected which transmit a narrow band of frequencies corresponding to the absorption peaks of the solution examined. In general the colour of the appropriate filter is complementary to that of the solution examined. Filters may be used between the light source and the cups, or mounted between the eyepiece and the observer's eye.

The calculation of the results is extremely simple provided the depth of colour is proportional to the amount of solute present. This is generally true when there is no great difference between the strengths of unknown and standard solutions, but no comparison should be attempted if the readings differ by more than 20%. Rather a stronger standard or greater dilutions of the unknown should be prepared.

The concentration of the substance, estimated in mg. per ml., of colorimeter solutions, is given by the expression :

$$\frac{\text{Depth of standard in millimetres}}{\text{Depth of unknown in millimetres}} \times \text{concentration of standard in mg. per 100 ml.}$$

Any dilutions must be compensated when working out the final strength.

An alternative procedure which simplifies the calculation may be adopted. The unknown is set at an arbitrary fixed position and the depth of the standard is varied. With the same provisions as mentioned above, the strength of the standard may then be chosen so that the colorimeter reading multiplied by an integral number gives the result directly in mg. per 100 ml. of sample.

The "Spekker" Photoelectric Absorptiometer

The use of photoelectric cells to measure the light absorption of solutions affords a convenient means of avoiding the personal error involved in visual matching. This principle has been employed in the design of the "Spekker" Photoelectric Absorptiometer.

As in the colorimeter, light from a common source is made to pass through the cells and filters before falling on two matched photoelectric surfaces, but, whereas in the colorimeter a comparison is made with a standard solution, in the absorptiometer a direct reading of optical density is made.

The diagrammatic sketch (see Fig. 40) shows the general arrangement of the apparatus. The light source, which consists of a 100 kW. metal vapour or filament lamp, occupies a central position, whilst the photoelectric surfaces are mounted on either

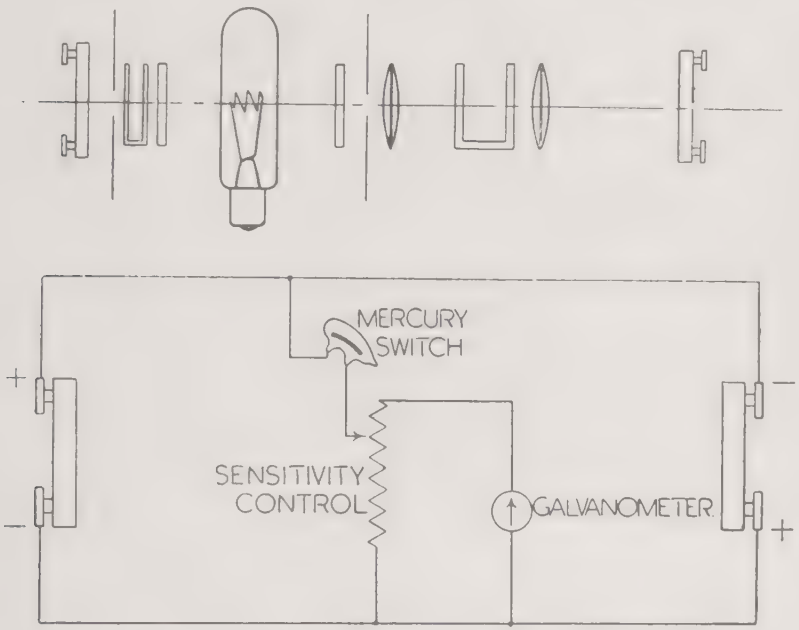

Fig. 40. Photoelectric Absorptiometer.

side; the "indicating" cell on the right, the "compensating" cell on the left.

Light, from the source towards the right-hand side, passes through a variable calibrated aperture, through the absorbing solution and is brought to a focus by a lens system on the indicating cell. As a direct result of this optical arrangement, the closing of the aperture reduces the intensity of light falling on the photosensitive surface, without reducing the area illuminated. The calibration of the aperture is a special feature of the instrument and the rotating drum control is graduated to measure

optical density, a function found to vary approximately in a linear manner with concentration.

To the left, the light, after passing through a protective cell containing solvent, falls on the compensating photoelectric cell. There is no lens system on this side, its place being taken by an iris diaphragm between the solvent cell and the photoelectric surface, which, being adjusted, varies the amount of light on the sensitive surface. The two photoelectric cells are connected in opposition (see Fig. 40) across a sensitive galvanometer which is used as a null point indicator.

Procedure. The procedures of calibrating the instrument and of measuring the absorption of an unknown solution are substantially the same. Three cells are required, two of which contain solvent; the third contains the absorbing solution. One of the cells containing solvent is placed on the left-hand side, that containing the solution on the right-hand side. The iris diaphragm is stoppered down to fullest extent and the drum-controlled aperture set in the zero position. The light is switched on and the galvanometer is set to maximum sensitivity (full scale deflection) by a control mounted on the right-hand side of the instrument. The iris diaphragm is then opened up until the pointer of the galvanometer returns to the zero position. A vernier control of the iris diaphragm makes it possible to carry out this adjustment with accuracy.

The second cell containing solvent is mounted on a sliding rack which is then moved into position so that the solvent takes the place of the absorbing solution. Light of greater intensity now falls on the indicating cell and the drum aperture must be stoppered down to restore the balance. The drum reading when the null point has been reached gives a measure of the optical density of the solution examined.

The instrument must be calibrated against standard solutions for each compound estimated, but following the initial calibration standard solutions are no longer necessary, except for checking the readings at long intervals. The symmetrical nature of the photoelectric circuit makes the reading independent of fluctuations of mains voltage and of variations in the sensitivity of galvanometer or photoelectric elements.

Many workers consider the procedure recommended above

somewhat tedious when many estimations of the same compound are being carried out simultaneously and prefer to use a simpler and more rapid method of taking readings. In this alternative procedure the drum reading is set at 1·0 and the so-called " water to water, one " balance is made, using solvent in both right and left cells. The right-hand cell is then replaced by one containing the absorbing solution and the drum aperture is then opened until the balance is restored. The method is subject to slight error if the photoelectric surfaces are fatigued by long exposure, but the error is negligible if the readings are made over a short interval of time and the additional precaution taken of checking the balance at frequent intervals.

The sensitivity of the instrument is, of course, greatly increased by the use of suitable filters. Not only is there an over-all gain in selectivity, but frequently the use of filters simplifies the relationship of calibration readings to concentration of solution.

BLOOD ANALYSES

The Collection and Storage of Blood Specimens

The first prerequisite of an accurate blood analysis is a representative blood sample. For a complete analysis the method of capillary puncture is unsuitable, for not only is it impossible to obtain the necessary volume, but it is difficult to avoid adulteration by tissue fluid. On the other hand, venipuncture affords a convenient method of withdrawing blood samples ample in volume for any series of estimations. The safety of the method depends upon the maintenance of aseptic conditions and in the main upon the sterilisation of the syringe.

Probably the most reliable method of sterilisation is by dry heat, whereby the syringe, ready mounted and lubricated with liquid paraffin, is maintained at 180° C. for not less than half an hour. The method has the dual advantage of convenience and reliability, but it is unfortunately not applicable to metal-bound syringes, nor can the needle be mounted on an all-glass syringe during the sterilising process. The sterilisation of syringe and needles under liquid paraffin at 180° C., although satisfactory, suffers from the same drawbacks. In any case, under no circum-

stances can a method involving the use of liquid paraffin be employed whenever fats or cholesterol are being estimated.

If no alternative is available, alcohol or ether may be employed. In each case the syringe should be flushed with sterile saline before use (to prevent clotting and hæmolysis).

The technique of blood withdrawal by venipuncture is a matter of common knowledge in the medical field. Not so well known, however, are the limitations set by chemical considerations, factors which, sometimes overlooked by the uninitiated, can vitiate the most careful analysis.

The blood received in the syringe should be transferred as quickly as possible to a small bottle or tube containing anticoagulant. A most frequent source of error lies in the choice of this anticoagulant, or indeed in making a decision whether an anticoagulant should be used at all. For most purposes requiring whole blood or plasma, neutral potassium oxalate at a final concentration of 2 mg. per ml. of blood is very satisfactory. For convenience, the oxalate is added to the tubes prior to use in the form of a solution ; 0·5 ml. of a 4% solution is sufficient for 10 ml. of blood. The solution is run over the sides of the tubes which are dried in the oven, leaving the oxalate distributed in a finely divided, easily soluble form.

Oxalate is not always suitable, and in calcium estimations it must never be used. The alternative anticoagulants, sodium fluoride and sodium citrate, are appropriate substitutes for oxalate only in certain estimations. For example, fluoride, a specific enzyme inhibitor, is useless for any estimation involving the use or assay of an enzyme (*e.g.*, urease in urea estimations or for the assay of phosphatase activity). Paradoxically, it is just this property of fluoride which makes it pre-eminently suitable for the collection of blood samples for inorganic phosphate estimations, where failure to inactivate the phosphatases of blood will lead to the liberation of organically bound phosphate. The use of fluoride to prevent glycolysis in normal blood is fully described in the appropriate section. If sodium citrate is used as an anticoagulant, the final concentration should be 5 to 7 mg. per ml.

For obvious reasons, all three anticoagulants, potassium oxalate, sodium fluoride and sodium citrate, are quite unsuitable when sodium and potassium are themselves being estimated and

it is the normal procedure to estimate these cations in serum. Calcium estimations, too, are frequently carried out on serum, although citrated blood may be used. If clotted blood is required, the specimen is transferred directly to a clean centrifuge tube and set aside until the clot contracts.

Partially hæmolysed specimens should always be treated with reserve. This is particularly so when estimating the serum or plasma content of any factor known to be present in much higher concentration in the erythrocytes. This source of error is well exemplified in the case of potassium assay, where slight hæmolysis will give a spurious result. Likewise, if serum phosphatase estimations are to be of diagnostic value, clear unhæmolysed blood must be obtained, since both alkaline and acid phosphatases are present in substantial concentrations in the erythrocytes.

In the methods subsequently described the nature and volume of the blood sample for each estimation has been recorded in the appropriate section. The following table gives normal values for those blood constituents which are most frequently determined.

Estimation	Whole Blood	Plasma or Serum
Carbonate (alkali reserve)	—	53–77 ml. CO_2 per 100 ml.
Calcium (serum)	—	9·9–11·1
Chlorides (as NaCl)	450–530	560–620
Cholesterol	170–220	170–220
Nitrogen Fractions :		
Total nitrogen	2·6–4·3 g.%	1·1–1·4 g.%
Non-protein nitrogen	20–40	18–30
Plasma Proteins :		
Total proteins	—	6·3–7·8 g.%
Albumin	—	4·5–5·2 g.%
Globulin	—	1·6–2·2 g.%
Fibrinogen	—	0·2–0·4 g.%
Phosphatase acid	—	less than 2·5 units
Phosphatase alkaline	—	3·5–13·5 units
Phosphorus inorganic (as P)	2·5–4·8	2·5–4·8
Potassium	150–250	17–22
Sodium (serum)	—	325–350
Sugar (fasting)	80–120	80–120
Urea	20–30	20–30
Uric acid	1·5–2·5	1·5–2·5

Values are in mg. per 100 ml. unless otherwise indicated.

The Preparation of a Protein-free Filtrate

Method of Folin and Wu (1)

In the methods which follow, the Folin and Wu filtrate is utilised for the determination of sugar, urea, non-protein nitrogen and creatinine.

Reagents. 1. 10% sodium tungstate. This should be quite clear and alkaline to phenolphthalein.

2. $\frac{2}{3}$ N. sulphuric acid.

Method. Suitable clean pipettes are selected, washed out with alcohol and ether and dried by air suction from a water pump. The following are then pipetted into a flask :—

One volume of blood.
Seven volumes of water.
One volume of the tungstate solution.
One volume of the acid.

The reagents should be allowed to run in drop by drop whilst the contents of the flask are gently agitated. When the addition is complete, the mixture is violently shaken and if the precipitation of the blood proteins has been complete, the colour changes in a few minutes from bright red to chocolate brown. If these conditions are satisfied, the resulting solution is filtered, using a minimum quantity of filter paper and pouring the whole of the fluid on to the funnel at once. The filtrate, which should be water clear, may be preserved almost indefinitely by the addition of a few drops of toluene.

The above method is used strictly as described only for sugar estimations. The following minor alteration is important in practice. Excluding the estimations of sugar and uric acid, where strict adherence to the order of adding reagents is important, it will be found advantageous to add the $\frac{2}{3}$ N. sulphuric acid prior to the tungstate solution. The acid *p*H of the solution before the addition of the tungstate causes a rapid formation of acid hæmatin and gives a protein-free filtrate which is not only obtained more quickly than in the original Folin and Wu method, but which is less prone to give cloudy colloidal solutions.

Creatinine Retention Test

Method of Barrett (2)

Experience has shown that high creatinine retention is frequently associated with increased non-protein nitrogen and urea levels. Much saving of time in an emergency and increase in accuracy may be gained if, before proceeding with the estimation of non-protein nitrogen, urea and creatinine, the following test is carried out. Whenever a positive result is encountered, smaller quantities of filtrate should be taken, thereby preventing ruined analyses due to high concentrations of these substances encountered unexpectedly.

Reagent. Add 25 ml. of 10% potassium iodide to 100 ml. of Nessler's reagent (see under Non-protein Nitrogen).

Method. Add 1 ml. of this reagent to 1 ml. of tungstic acid filtrate in a small tube. Observe the tube against a blank background at a temperature of 15° to 20° C. for one minute. If the test is positive a greyish-white turbidity will appear in forty seconds.

Interpretation. A positive result indicates a creatinine content of over 2 to 3 mg. per 100 ml. in the blood. Blood preserved with formalin cannot be used for this test.

Estimation of Non-protein Nitrogen

Modification of Method of Folin and Wu (1)

Reagents. 1. Digestion mixture. The following mixture has proved satisfactory and has the advantage of leaving the tubes unattacked even after prolonged heating :—

100 ml. of concentrated nitrogen-free sulphuric acid (added last).
0·25 g. of sodium selenate.
3 ml. of syrupy phosphoric acid.
150 ml. of water.

2. Standard ammonium sulphate solution containing 0·1 mg. of nitrogen per ml. (0·4716 g. of specially purified ammonium sulphate per litre). After the addition of two drops of strong sulphuric acid to prevent the growth of green moulds, dilute to one litre.

3. Nessler's reagent (Koch and McMeakin (3)). Dissolve 22·5 g. of iodine in 20 ml. of water containing 30 g. of potassium iodide. Add 30 g. of mercury and shake the mixture, keeping it cool in cold water until the supernatant liquid has lost its yellow colour. Decant the top liquid into a 200 ml. flask and test a drop of the solution with 1% soluble starch. A faint blue colour should develop. If no colour appears add a few drops of iodine solution of the above strength until a faint excess of iodine can be detected. Dilute to 200 ml. and pour into 975 ml. of 10% sodium hydroxide solution. Mix and allow to clear by standing.

Method. Monax or Pyrex tubes (8 × 1 in.) are used, in the bottoms of which small pieces of platinum wire have been sealed to prevent bumping. Pipette into the tube the following volumes :—

5 ml. of protein-free blood filtrate,
1 ml. of the acid digestion mixture,

and heat the tube over a sand bath or a microburner. After several minutes' steady boiling, the concentrated residue darkens and when only 0·5 ml. remains, dense white fumes fill the tube. When this stage is reached cover the mouth of the tube with a watch glass. Continue heating for several minutes after the liquid becomes colourless, allowing the acid to wash down the walls of the tube. A precipitate of tungstic acid sometimes appears at this stage, but this will dissolve during the subsequent operations. Allow the tube to cool, add not more than 30 ml. of water and cool the contents of the tube by immersion in cold water. Now add rapidly 15 ml. of Nessler's reagent from a burette, whilst rotating the liquid in the tube by means of a twirling movement. Dilute to the 50 ml. mark with water, mix the contents of the tube and compare with the standard in the colorimeter.

Preparation of the Standard. Introduce the following into a similar hard glass tube graduated at 50 ml.

1·5 ml. of standard ammonium sulphate solution.
1 ml. of acid digestion mixture.
30 ml. of water.
15 ml. of Nessler's reagent.

Dilute to the 50 ml. mark, mix well and set at 20 mm. in the colorimeter. Use a blue filter to facilitate matching.

Calculation. Six hundred, divided by the colorimeter reading, gives the result in milligrams per 100 ml.

Estimation of Blood Urea

Modification of the Method of Barrett (4)

This direct Nesslerisation method is rapid and has proved useful in clinical work.

Reagents. 1. Citrate buffer solution. Dissolve 60 g. of sodium citrate in about 180 ml. of water. Add 1 ml. of glacial acetic acid and dilute to 200 ml.

2. Urease solution. Suspend 10 g. of "Arlco" jack bean meal in 125 ml. of water and add 5 ml. N/10 HCl. Filter after several minutes. Pipette 50 ml. of the clear filtrate into a centrifuge tube and add 1 ml. of 10% acetic acid. Spin down the precipitated urease for ten minutes. Decant the supernatant fluid and allow the tube to drain. Dissolve the precipitate in 5 ml. of the citrate buffer solution and dilute to 50 ml. Preserve in the refrigerator.

3. Nessler's reagent (see under Non-protein Nitrogen).

4. Standard ammonium sulphate solution. This is prepared by making an accurate 1 in 10 dilution of the ammonium sulphate referred to under Non-protein Nitrogen, and contains 0·01 mg. of nitrogen per ml.

5. Sodium hypochlorite solution containing 10% of available chloride.

6. 2% phosphotungstic acid in 0·2 N. sulphuric acid.

Method. Add 1 ml. of urease solution to 5 ml. of protein-free blood filtrate in a 15 ml. centrifuge tube. Incubate at 50° C. for ten minutes. Add 1 ml. of phosphotungstic acid, mix well, and centrifuge for a few seconds to sediment the precipitate. Transfer 5 ml. of the clear supernatant fluid to a 6 in. by 1 in. test tube containing 10 ml. of distilled water. Prepare the Nessler-hypochlorite mixture by adding three drops of sodium hypochlorite solution to 20 ml. of Nessler's reagent. Add 2 ml. of this mixture to the test tube, twirling the tube to rotate the contents during the addition. Compare in the colorimeter with the standard set at 20 mm., using a blue filter to facilitate matching.

Standard. Introduce 5 ml. of standard ammonium sulphate,

10 ml. of water and 2 ml. of Nessler-hypochlorite reagent into a 6 in. by 1 in. test tube.

Calculation. Six hundred, divided by the reading, gives the result in milligrams of urea per 100 ml.

Estimation of Proteins in Plasma

Modification of Method of Hawk and Bergeim (5)

3 ml. plasma.

Reagents. 1. 22·2% sodium sulphate. This is prepared at 37° C. and stored in the 37° C. incubator to prevent crystallisation of the salt.

2. 2·5% calcium chloride solution.

3. 0·9% sodium chloride solution.

4. Solution of 1 part 2·5% calcium chloride added to 50 parts 0·9% sodium chloride.

5. Digestion mixture (see Non-Protein Nitrogen).

6. Nessler's reagent (see Non-Protein Nitrogen).

7. Standard ammonium sulphate solution (see Non-Protein Nitrogen) calibrated at 50 ml.

8. Citrate buffer (see Non-Protein Nitrogen).

Method. *Fibrinogen.* Pipette 0·5 ml. of plasma into a test tube graduated at 25 ml. Dilute to the 25 ml. graduation mark with the sodium chloride calcium chloride mixture, mix and allow to stand for half an hour or until the fibrin clot separates. Rotate the tube gently until the fibrin adheres together and filter through a hardened filter paper (9·0 cm. Whatman No. 54). Discard the majority of the filtrate, to wash out ammonium compounds contained in the paper, and collect the last few ml. of liquid. Open the filter paper and unite the particles of fibrin. Press the clot between dry filter papers and drop it into an 8 in. by 1 in. tube calibrated at 50 ml. Proceed as described under "digestion."

Albumin and Globulin and Non-Protein Nitrogen. 1 ml. of filtrate from the fibrinogen precipitation is transferred to an 8 in. by 1 in. tube for "digestion."

Albumin and Non-Protein Nitrogen. Place 0·5 ml. of plasma in a 6 in. by $\frac{3}{4}$ in. tube and add 15 ml. of 22·2% sodium sulphate solution. Stopper, mix well and incubate at 37° C. for three

hours or overnight. Filter in the incubator using a double ammonia-free paper (9·0 cm. Whatman No. 1). Take 1 ml. of *clear* filtrate in an 8 in. by 1 in. tube for "digestion."

Non-Protein Nitrogen. Analysis carried out by the usual method.

Digestion. The digestions are carried out in hard glass tubes (8 in. by 1 in.) into the bottoms of which small pieces of platinum wire have been fused. 1 ml. of digestion mixture is added to each tube. Heat the tubes on a sand bath and when white fumes appear watch glasses are used to cover the mouths of the tubes. Continue heating until the liquid becomes colourless, washing down any particles of undigested matter with the hot acid. After cooling, wash the lower surfaces of the watch-glasses with distilled water, collecting the washings in the appropriate tubes and adding about 30 ml. of water in all. Add 1 drop of citrate buffer to each tube. Cool to room temperature. Add 15 ml. of Nessler's reagent to each tube, dilute with water to 50 ml. and mix. Compare in a colorimeter with standard prepared from 1·5 ml. standard ammonium sulphate solution, 1 ml. of digestion mixture, 1 drop of citrate buffer, water and 15 ml. of Nessler's reagent, diluting to 50 ml.

If a high fibrin result is encountered, a stronger standard should be prepared, or the fibrin digest should be diluted prior to Nesslerisation.

Calculation. Standard set at 20 mm.

Fibrinogen : $\dfrac{3{\cdot}75}{R_1} = \text{g. per 100 ml.}$

Albumin : $\left(\dfrac{9{,}300}{R_2} - \text{N.P.N. in mg./100 ml.}\right) \times \dfrac{6{\cdot}25}{1{,}000} = \text{g./100 ml.}$

Globulin + Albumin : $\left(\dfrac{15{,}000}{R_3} - \text{N.P.N. in mg./100 ml.}\right) \times \dfrac{6{\cdot}25}{1{,}000} = \text{g./100 ml.}$

N.P.N. is the non-protein nitrogen in mg. per 100 ml. of plasma. R_1 is the reading of the fibrin digest, R_2 and R_3 those of the

albumin and albumin plus globulin digests respectively. The value for globulin is obtained by subtracting the albumin figure from the albumin plus globulin figure. The use of tables for calculation of the final results is recommended when many analyses are being carried out.

Estimation of Prothrombin

Fullerton's Modification of Quick's Method (6)

4·5 ml. of blood collected in 0·5 ml. of 1·34% sodium oxalate solution. 4·5 ml. of normal blood collected in 0·5 ml. of 1·34% sodium oxalate solution.

Reagents. 1. Sodium oxalate solution. Dissolve 1·34 g. of pure sodium oxalate in 100 ml. of distilled water.

2. Calcium chloride solution. Dissolve 1·11 g. of anhydrous calcium chloride in 100 ml. of distilled water.

3. Source of lecithin. Phospholipid of blood plasma sample collected.

4. Source of thromboplastin. 0·1 mg. of Russell viper venom diluted to 1 ml. prior to use.

Principle. Two stages are recognised in the clotting process :

1. Prothrombin plus calcium plus thromboplastin forms thrombin.

2. Thrombin plus fibrinogen forms fibrin.

The absolute amounts of Russell viper venom and of calcium are kept constant and under these circumstances the rate of clotting is related to the concentration of prothrombin. The time for clotting to take place in the sample is compared with the time required for a normal sample of blood under identical conditions.

Method. 4·5 ml. of a normal blood sample are collected in a centrifuge tube containing 0·5 ml. of 1·34% sodium oxalate solution. After the cells have been spun down, 0·2 ml. of plasma is removed and mixed with 0·2 ml. of the diluted Russell viper venom. The calcium solution and the plasma mixture are maintained at 37° C. and, thirty seconds after the initial mixing, 0·2 ml. of the calcium solution are added. The tube, still maintained at 37° C., is shaken and the number of seconds before the appearance of a fibrin clot is noted.

An exactly similar determination is carried out using the experimental sample. The times, in seconds, of the normal and experimental sample are reported.

Estimation of Alkaline Phosphatase

Method of King and Armstrong (7)

1 ml. serum or plasma.

Reagents. 1. Buffer-substrate solution. N/200 sodium phenyl phosphate in N/20 veronal.

10·3 g. sodium veronal.
1·09 g. disodium phenylphosphate.
Water to 1 litre.

Add a few drops of chloroform as preservative and store in a well-stoppered bottle in a refrigerator.

2. Phenol-colour reagent (Folin and Ciocalteu (8)). 100 g. sodium tungstate and 25 g. sodium molybdate are added to 70 ml. of water contained in a 2 litre round-bottomed flask. To the resultant mixture add 50 ml. 85% phosphoric acid, 100 ml. concentrated hydrochloric acid and reflux for ten hours. 150 g. of lithium sulphate are then added, together with 50 ml. of water and a few drops of bromine. The reagent is boiled without condenser until the excess bromine has been driven off and the final solution has acquired a yellow-green coloration. Cool and dilute to 1 litre. The reagent is further diluted 1 to 3 prior to use.

3. Sodium carbonate solution. 12·5 g. anhydrous sodium carbonate per 100 ml.

4. Stock phenol solution. Dissolve slightly more than 1 g. of phenol in 1 litre of $\frac{N}{10}$ hydrochloric acid. Transfer 25 ml. of the solution to a separate flask, add 50 ml. of $\frac{N}{10}$ sodium hydroxide and heat to 65° C. Pipette into the alkaline phenol solution 25 ml. of $\frac{N}{10}$ iodine solution, stopper and allow to stand for forty minutes at room temperature. Acidify with 5 ml. of concentrated

hydrochloric acid and back-titrate the excess iodine with $\frac{N}{10}$ sodium thiosulphate solution.

Each ml. of iodine is equivalent to 1·567 mg. of phenol. On this basis dilute the remainder of the original phenol solution so that 1 ml. of stock phenol solution contains 1 mg. of phenol.

5. Standard phenol colour reagent. Transfer 1 ml. (1 mg.) of stock phenol solution to a 100 ml. graduated flask, add 25 ml. of dilute phenol colour-reagent and make up to 100 ml. This reagent should be prepared as required but may be stored up to one week in a refrigerator.

Method. It is important that cell-free unhæmolysed serum or plasma be used.

Pipette 10 ml. of buffer-substrate solution into a tube (6 in. by 1 in.) and place in a water bath at 37·5° C. for five minutes or more. Without removing the tube from the bath, add exactly 0·5 ml. of sample, stopper, mix and incubate at 37·5° C. for exactly thirty minutes. At the end of this time add 4·5 ml. of dilute phenol-colour reagent to precipitate the protein and stop the enzymic reaction.

The control solution is prepared in a similar manner as follows : Pipette 10 ml. of buffer-substrate into a tube (6 in. by 1 in.). Add 0·5 ml. serum and immediately 4·5 ml. of dilute phenol-colour reagent.

Filter both test and control solutions and pipette 10 ml. of each filtrate into two clean tubes. Add 4 ml. of 12·5% sodium carbonate solution, mix and replace both tubes in the water bath for fifteen minutes to develop the colour.

The colorimeter comparison is made against a standard made up simultaneously with the test and control solutions. 4 ml. of 12·5% sodium carbonate solution are added to 10 ml. of standard phenol reagent and the mixture placed in the water bath along with the test and control solutions for fifteen minutes at 37·5° C.

Comparison and Calculation. In phosphatase estimations the unknown solution is set at 30 mm. and the depth of the standard solution is varied. In turn the standard solution is read against the test solution and then against the control solution. The units of phosphatase activity per 100 ml. equal the reading of the standard against test, minus the reading of standard against

control and the result so obtained is numerically equal to the mg. of phenol which would be set free from the substrate by 100 ml. of sample under the standard conditions.

Procedure if Enzyme Content is Very High. If more than 30 but less than 60 units of phosphatase are present, the reading of the unknown for the test solution may be set at 15 mm. When more than 60 units are present, the phosphate produced has a retarding influence on the velocity of the hydrolysis and the serum should then be suitably diluted with normal saline before testing.

Estimation of Acid Phosphatase

Method of Gutman and Gutman (9)

1 ml. serum or plasma.

Reagents. 1. Substrate 0·005 M. monophenyl phosphate. Dissolve 1·09 g. of disodium phenyl phosphate in 500 ml. water.

2. Buffer. 0·1 M citrate *p*H 4·9. Dissolve 42 g. of crystalline citric acid (mono hydrate) in water, add 376 ml. of N sodium hydroxide and make up to 1 litre. Adjust the *p*H to 4·9 with sodium hydroxide or hydrochloric acid as required. Preserve with a few drops of chloroform in well-stoppered bottles in the refrigerator.

3. Buffer-substrate is prepared immediately before use by mixing equal volumes of reagents 1 and 2.

4. Phenol-colour reagent (see Alkaline Phosphatase).

5. Sodium carbonate solution (see Alkaline Phosphatase).

6. Stock phenol solution (see Alkaline Phosphatase).

7. Standard phenol reagent (see Alkaline Phosphatase).

Method. The method follows that already described under Alkaline Phosphatase, except in two important respects : (1) Until actually used, the buffer and substrate solutions are stored separately ; (2) an incubation period of three hours is necessary to give satisfactory colorimeter readings.

Calculation. The number of units of phosphatase activity per 100 ml. is equal to the reading of standard against test, minus the reading of standard against control, divided by the number of hours of hydrolysis.

Estimation of Abnormal Blood Pigments

Hartridge Reversion Spectroscope (10)

Identification of carboxyhæmoglobin, sulphæmoglobin and methæmoglobin.

Principle. This instrument is a development of the direct vision spectroscope designed to facilitate accurate measurement

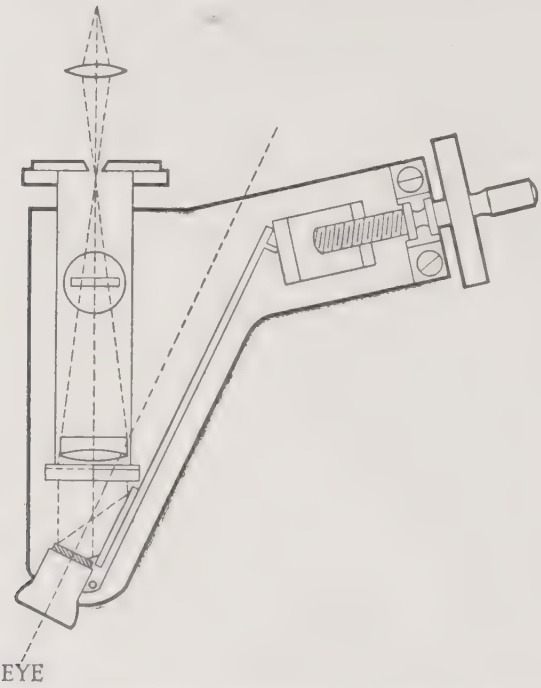

Fig. 41. Reversion Spectroscope (*after Hartridge*).

of absorption and emission spectral bands. As in the simpler instrument, the spectra are produced by an optical system which gives chromatic dispersal with minimum deviation. The optical system (shown in Fig. 41) is such that from a common slit source two spectra are produced simultaneously, one above the other, but with the colour order reversed. One spectrum is fixed in position, the other may be moved relative to the fixed spectrum by means of a vernier screw. This screw adjustment is calibrated directly in units of wave length (Angström units or $\mu\,\mu$).

Method. Before use it is a wise precaution to check the calibration of the instrument. For this purpose a neon lamp is

provided as a standard fitting of the instrument. This neon lamp used in place of the normal white light source produces an emission spectrum with four characteristic, easily recognised lines at 4358·3 Å.U., 5460·7 Å.U., 5852·5 Å.U. and 6402·2 Å.U. respectively. The observer, looking through the instrument towards the neon lamp source, sees two parallel spectra and by means of the vernier screw adjustment may alter their relative positions. Selecting one emission line, say 4358·3 Å.U., the relative position of the two spectra is adjusted until this same line in both spectra is colinear. The vernier reading of the instrument should then agree with the accepted wave length of the selected line. The position of each of the four lines is checked in turn. The neon lamp is replaced by the white light source and the instrument is ready for use.

The spectrum seen when the instrument is used against a source of white light is normal, with general emission at all wave lengths from red to violet. In use, diluted blood contained in glass cells is placed between the light source and the objective of the instrument. In consequence the emergent light viewed by the observer is deficient in certain wavebands which appear as dark absorption bands in the spectra. As the light source is common to both images, the same absorption bands appear in both spectra and any absorption band may be made colinear with its counterpart in the inverted spectrum.

Qualitative Tests

Carboxyhæmoglobin. 1 ml. whole blood sample and 1 ml. normal whole blood.

Normal blood and the sample to be examined for abnormal pigment are laked by dilution with 1% ammonia solution, 1 in 50 to 1 in 200 according to experience. These diluted samples are placed respectively in two cells which are mounted on a sliding rack, so arranged that each cell in turn may be examined. The normal sample is first examined and the "α" bands (5,780 Å. for oxyhæmoglobin) of both spectra are aligned. This adjustment is greatly facilitated by careful focussing and by adjustment of the slit width.

The sliding rack is then adjusted to bring the test sample into

position, care being taken that the adjustment of the instrument is not disturbed. If carboxyhæmoglobin is present, there is a slight movement of the α band towards the violet in both spectra and the lines will no longer be colinear. The band shift for 100% conversion to carboxyhæmoglobin is only 60 Å.

Methæmoglobin and Sulphæmoglobin. 1 to 5 ml. whole blood.

As distinct from the normal blood pigments, methæmoglobin and sulphæmoglobin have an additional "α" absorption band in the red region of the spectrum—methæmoglobin 6,300 Å. and sulphæmoglobin 6,180 Å. Except in advanced cases, when the blood or plasma acquires a brown tint, there is no obvious divergence from the colour of normal blood. The bands are not pronounced and, having approximately one-tenth of the intensity of the "α" line of oxyhæmoglobin, may only be observed with relatively wide absorption cells and low dilutions of blood or plasma.

The technique of identifying these two abnormal pigments is broadly similar to that already described for carboxyhæmoglobin. For comparison purposes, artificial solutions of methæmoglobin and sulphæmoglobin are necessary. The methæmoglobin solution may be obtained by adding a crystal of potassium ferricyanide to 10 ml. of a 1 in 100 dilution of normal blood ; the sulphæmoglobin by adding 0·1 ml. of 0·1% phenylhydrazine hydrochloride followed by 1 drop of saturated H_2S water to a similar blood dilution. These solutions should be prepared immediately prior to use.

The instrument, with the patient's blood sample in position, is set so that the suspected "α" bands in the red region of the spectrum are colinear. In turn, without altering the setting of the instrument, solutions of methæmoglobin and sulphæmoglobin are substituted and the "α" lines examined for linearity. It is manifest that the artificially prepared pigment which on examintion produces no line "shift" must be the abnormal pigment present in the examined blood sample.

Spectroscopic examination of the pigments, taken in conjunction with their differential chemical behaviour, affords more reliable information. Reducing agents, such as ammonium sulphide or sodium hydrosulphite, have no effect upon sulphæmoglobin but reduce methæmoglobin to hæmoglobin with the

consequent disappearance of the absorption band at 6,300 Å. Concentrated ammonia (0·2 ml. per ml. blood) and 1% cyanide will also cause the rapid disappearance of the "α" line of methæmoglobin. The latter reagent will also reduce sulphæmoglobin to cyanhæmoglobin with the consequent disappearance of the characteristic line, but the process is extremely slow. Furthermore, on passing coal gas through a solution of sulphæmoglobin, there is a perceptible movement of the "α" line towards the violet end of the spectrum. The movement is not great but is entirely absent in the case of methæmoglobin.

Estimation of Blood Sugar

Method of Folin and Wu Modified by Benedict (11)

1 ml. whole blood collected in fluoride.

The glycolysis which takes place in oxalated blood cannot be neglected. Whilst estimations carried out on samples immediately after withdrawal are unlikely to be affected by glycolysis, the storage of samples, as when left to the end of a glucose tolerance test, introduces errors which may materially alter the sugar response curve. Under these circumstances, each sample should be collected into a tube containing 20 mg. potassium oxalate and 7 mg. sodium fluoride. This anticoagulant is sufficient for 10 ml. of blood and will inhibit glycolysis for two or three days. The method described below gives substantially "true" sugar values, the reducing power of non-glucose substances being suppressed by the use of alanine and sodium bisulphite.

Reagents. 1. Copper reagent. Dissolve 15 g. of anhydrous sodium carbonate, 3 g. of alanine and 2 g. of Rochelle salt in about 250 ml. of distilled water. Dissolve 3 g. of crystalline copper sulphate in about 100 ml. of distilled water and add this solution with stirring to the previous solution. Dilute to 500 ml. and mix. This solution will keep for six to eight weeks in the refrigerator.

2. 1% sodium bisulphite. The solution should be prepared each month.

3. Copper bisulphite reagent. Mix 1 volume of sodium bisulphite solution with 20 volumes of copper reagent. This mixture is prepared as required.

4. Phosphomolybdic acid. To 133·3 g. of ammonia-free molybdenum trioxide (AR) and 75 g. of anhydrous sodium carbonate in a large Erlenmeyer flask, add 500 ml. of water in small portions while shaking. Heat to boiling and filter off the insoluble residue. Wash the residue on the paper until the total volume of filtrate and washings is 600 ml. Add 300 ml. of 85% phosphoric acid and dilute to 1 litre.

5. Standard glucose solutions. 1 g. of pure anhydrous glucose is dissolved in about 50 ml. of saturated benzoic acid solution and diluted to 100 ml. with benzoic acid solution. One ml. of this solution made up to 100 ml. will serve as a standard for most bloods, but it is well to have a double standard ready in case a high blood sugar is encountered. This is made by diluting 2 ml. of the stock solution to 100 ml. All dilutions are made with saturated benzoic acid solution. The dilute standards should be prepared at frequent intervals (four weeks).

FIG. 42. Folin's Blood Sugar Tube.

Method. Two ml. of filtrate and 2 ml. of the standards are transferred to special sugar tubes (see Fig. 42). Add 2 ml. of the copper bisulphite reagent to each tube. Mix the contents of the tubes by shaking laterally and place the tubes in boiling water for five and a half to six minutes. Place the tubes in cold running water for one to two minutes and add 2 ml. of the phosphomolybdic colour reagent. Mix the solutions and after one to two minutes dilute to the 25 ml. mark with water. After mixing, the unknown is compared in the colorimeter with the appropriate standard which is set at 20 mm. The reading should be taken within ten minutes of adding the colour reagent.

Calculation. Two thousand for the low standard and 4,000 for the high standard, divided by the colorimetric reading, gives the sugar content in milligrams per 100 ml.

Estimation of Sulphonamide Drugs

Method of Gardner and Dunn (12)

2 ml. whole blood.

Reagents. 1. 15% trichloracetic acid.

2. 0·1% sodium nitrite solution, freshly prepared.

3. 0·5% ammonium sulphamate solution.

4. 0·05% saponin solution.

5. 4 N. hydrochloric acid.

6. 0·1% N-(1-naphthyl)-ethylenediamine dihydrochloride in water. This solution should be kept in a dark bottle.

7. A stock solution of the sulphonamide estimated containing 200 mg. per litre. Five ml. of this solution are mixed with 18 ml. of 15% trichloracetic acid and the solution is diluted to 100 ml. to produce a standard containing 1 mg. of the drug per 100 ml.

8. Standard sulphonamide solutions. As the intensity of colour is not directly proportional to the quantity of drug present, it is necessary to prepare several standards to cover the desired range. These are prepared from the solution containing 1 mg. of drug per 100 ml. by taking the appropriate volume of this solution and diluting to 10 ml. with a solution containing 18 ml. of 15% trichloracetic acid per 100 ml. (2·7% trichloracetic acid). The working standards so obtained are treated in the manner described for blood filtrate.

Method. Two ml. of fluid containing drug are measured into a flask, diluted with 30 ml. of saponin solution and after one to two minutes precipitated with 8 ml. trichloracetic acid. After standing for five minutes the mixture is centrifuged.

Free Sulphonamide. To 10 ml. of the clear centrifugate 1 ml. of sodium nitrite solution is added. After three minutes excess of nitrite is removed by the addition of 1 ml. of ammonium sulphamate and after a further three minutes 1 ml. of the N-(1-naphthyl)-ethylenediamine dihydrochloride is added to the resultant solution. The colour produced is either measured in a Hilger Absorptiometer or compared against an appropriate standard in a colorimeter.

Total Sulphonamide. Ten ml. of the supernatant liquid are treated with 0·5 ml. of 4 N. HCl and the mixture is heated in a loosely stoppered tube in a boiling water bath for one hour.

After cooling the volume is made up to 10 ml. and the solution is treated as above.

The reagent blank on distilled water is low, but increases with time if the solution is left in the light, so that solutions should be protected from the light unless read immediately.

Calculation. $\frac{4{,}000}{R} \times$ mg. drug in 10 ml. working standard = mg. drug per 100 ml. blood.

Estimation of Cholesterol

Method of Reinhold and Shiels (13)

1 ml. whole blood or plasma.

Reagents. 1. Anhydrous sodium sulphate.

2. Acetic anhydride—sulphuric acid mixture. One volume of concentrated sulphuric acid is added, with shaking to 20 volumes of acetic anhydride. This mixture is freshly prepared as required.

3. Cholesterol solution, 1 mg. per ml. Dissolve 0·1 g. of cholesterol in 100 ml. of chloroform. Store in a dark stoppered bottle.

Method. All reagents and apparatus used throughout this estimation must be perfectly dry. One ml. of blood or plasma is pipetted into a small mortar containing about 8 g. of anhydrous sodium sulphate. It is then uniformly mixed, dried at 105° C. for ten minutes, cooled in a desiccator and pulverised. The powder is then transferred to a paper extraction thimble, inserted into a Soxhlet extraction thimble holder and extracted for two hours with chloroform (25 ml.). Interrupting the extraction whilst the Soxhlet thimble is full of pure chloroform, the chloroform extract is allowed to cool and transferred quantitatively to a stoppered tube graduated at 15 ml. The volume is made up to 15 ml. with pure chloroform.

The standard is prepared at the same time by pipetting 2 ml. of cholesterol standard (2 mg.) into a similar tube and diluting to 15 ml. with chloroform. Six ml. of freshly prepared acetic anhydride mixture are added to each tube and the contents are mixed. The solutions are then kept at 18° C. for forty-five minutes.

The standard is set at 20 mm. in the colorimeter and the reading of the unknown taken.

Calculation. $\frac{4{,}000}{\text{Reading}}$ = mg. of cholesterol per 100 ml.

Estimation of Calcium

Method of Clark and Collip (14)

2 ml. serum or citrated plasma.

Reagents. 1. $\frac{N}{100}$ potassium permanganate solution prepared as required by diluting a $\frac{N}{10}$ permanganate solution, standardised against an accurate $\frac{N}{10}$ sodium oxalate solution.

2. 4% ammonium oxalate solution.

3. Ammonia solution, prepared by diluting 2 ml. of concentrated ammonia (sp. gr. 0·880) to 100 ml. with distilled water.

Method. The principle of this method consists in precipitating the calcium as oxalate. The precipitate after washing and dissolving in sulphuric acid is titrated with permanganate.

Two ml. of clear serum or citrated plasma are measured into a 15 ml. centrifuge tube containing 2 ml. of water; 1 ml. of 4% ammonium oxalate solution is then added and mixed by tapping the tube. The mixture is then allowed to stand for at least thirty minutes, when it is again mixed and centrifuged for five minutes. The supernatant fluid is then removed by decantation and the tube drained for five minutes.

1. Three ml. of dilute ammonia are then added from a graduated pipette in such a way that the sides of the tube are washed down and the precipitate well mixed. The tube is then centrifuged and drained as before. The calcium oxalate precipitate is well mixed with about 0·3 ml of distilled water.

2. One ml. of $\frac{2}{3}$ N. sulphuric acid is added to dissolve the precipitate. The solution is titrated with $\frac{N}{100}$ potassium permanganate to a definite pink colour which must persist for at least one minute. During the titration the temperature should

not fall below 60° C. An incandescent lamp immersed in a vessel acting as a water bath not only maintains the necessary temperature, but increases the accuracy with which the end point can be read.

3. A microburette graduated in 0·01 ml. should be used for this titration.

Calculation. The number of ml. of permanganate used, multiplied by ten, will equal the number of mg. of calcium in 100 ml. of serum. The amount of $\frac{N}{100}$ permanganate needed to impart a definite pink colour to 2 ml. of fluid must be determined and subtracted from the quantity of permanganate used in the final titration.

Estimation of Potassium

Modification of Method of Kramer (15)

1 ml. of serum.

Reagents. 1. Sodium cobaltinitrite reagent :

(*a*) 25 g. of cobalt nitrate crystals are dissolved in 50 ml. water. 12·5 ml. of glacial acetic acid are then added.

(*b*) 120 g. of sodium nitrite are dissolved in 180 ml. water.

To solution (*a*) add 210 ml. of solution (*b*). Air is drawn through the mixture until the nitrous fumes are removed. The reagent is preserved in the refrigerator and filtered before using.

2. 4 N. sulphuric acid.

3. $\frac{N}{50}$ potassium permanganate (freshly prepared from $\frac{N}{10}$).

4. $\frac{N}{100}$ sodium oxalate (freshly prepared from $\frac{N}{10}$).

5. Standard potassium solution :

Stock : 8·914 g. K_2SO_4 per litre = 4 mg. K per ml.

Standard : Prepared as required by diluting 5 ml. of stock (4 mg.) to 100 ml.

Method. Separate the serum from the cells as soon as possible. Hæmolysed serum is not suitable.

Pipette 1 ml. of serum and 1 ml. of standard potassium solution into centrifuge tubes, previously cleaned by immersing in cleaning mixture and thorough rinsing with distilled water. Add slowly,

drop by drop, with shaking, 2 ml. of cobaltinitrite reagent to each tube. Allow to stand for thirty-five to forty minutes at room temperature and centrifuge for ten minutes. Rapidly decant the supernatant fluid and remove as much as possible of the liquid on the sides of the tubes with filter paper. Wash down the sides of the tubes with 5 ml. of wash water without disturbing the precipitate. Centrifuge and drain as before. Repeat the washing once more. Then add 1 ml. of 4 N H_2SO_4 and 2 ml. of $\frac{N}{50}$ $KMnO_4$ to each tube. Stir the precipitate with a finely pointed glass rod and immerse the tubes in boiling water for fifty to sixty seconds. Add 2 ml. of $\frac{N}{100}$ sodium oxalate *at once*. Then titrate to a faint permanent pink end with $\frac{N}{50}$ $KMnO_4$ from a 2 ml. microburette.

Calculation.

$$\frac{\text{No. of ml. of } \frac{N}{100} KMnO_4 \text{ used for unknown} \times 20}{\text{No. of ml. of } \frac{N}{100} KMnO_4 \text{ used for standard}}$$

equals mg. K per 100 ml.

No. of ml. of $\frac{N}{100}$ $KMnO_4$ used equals (2 × No. of ml. of $\frac{N}{50}$ $KMnO_4$ added in all) minus 2.

Colorimetric Determination of Serum Sodium

Modification of Method of McCance and Shipp (16)

1 ml. serum.

Reagents. 1. Alcohol zinc uranyl acetate solution saturated with "triple acetate." Dissolve 50 g. of uranyl acetate ($UO_2(CH_3COO)_2$ $2H_2O$) in 250 ml. of boiling 4% v/v acetic acid. Dissolve 150 g. of zinc acetate, ($Zn(CH_3COO)_2$ $2H_2O$) in 250 ml. of boiling 2% v/v acetic acid. Mix the two solutions whilst boiling. Bring the mixture just to the boil again. Stand overnight and filter if necessary. To the filtrate add an equal volume of absolute alcohol. Stand for forty-eight hours at 0° C., filter, and then store at 0° C. in a glass-stoppered bottle until required,

and filter or centrifuge enough for the test(s) just before use to remove suspended sodium zinc uranyl acetate.

There is usually sufficient sodium salt as impurity in the solution of acetates to yield more than enough "triple acetate" to saturate the reagent. If not, "triple acetate" must be added to saturate.

2. 95% alcohol saturated with "triple acetate." Add about 50 ml. of the above alcoholic zinc uranyl acetate solution to about 100 ml. of saturated NaCl in 50% alcohol. Allow to stand. Decant most of the supernatant fluid. Centrifuge and wash the yellow precipitate of "triple acetate" thoroughly with 95% alcohol. Suspend the washed precipitate in 95% alcohol, transfer to a glass-stoppered bottle with more of the alcohol to a total of about 500 ml. Shake thoroughly and store at 0° C. There must always be a precipitate of excess "triple acetate" at the bottom of the bottle. Centrifuge or filter enough for the test(s) just before use.

3. Stock NaCl, 0·89% (≡ 0·35% Na) made from pure dry analytical reagent.

4. Standard NaCl, 0·178% (≡ 0·07% Na) prepared from the stock NaCl by diluting 5 ml. with water to 25 ml. Therefore 1 ml. of standard solution ≡ 0·7 mg. of Na.

5. 20% trichloracetic acid solution (A.R.).

6. 20% potassium ferrocyanide solution (A.R.).

Method. The proteins are precipitated by trichloracetic acid. The sodium in an aliquot part of the supernatant fluid is precipitated as sodium zinc uranyl acetate ("Triple Acetate"). The precipitate is washed and treated with potassium ferrocyanide solution. The resulting plum-red colour (due to uranyl ferrocyanide) is compared in a colorimeter with a standard NaCl solution which has been treated similarly and simultaneously. Phosphates are not removed as the amount present in serum is insignificant.

Pipette 1 ml. of serum into a centrifuge tube containing 3 ml. of water and add whilst shaking 1 ml. of 20% trichloracetic acid solution. Centrifuge or filter to remove the precipitated proteins.

Pipette 1 ml. of filtrate (≡ 0·2 ml. serum) into a second centrifuge tube and add 10 ml. alcoholic zinc uranyl acetate solution.

Stir with fine glass rod until the yellow precipitate of sodium zinc uranyl acetate forms and wash the rod with a few drops

of alcohol zinc uranyl solution. Stand at 0° C. for one hour. Centrifuge well and drain off the supernatant fluid completely by inverting and wiping the mouth of the tube with filter paper. Add 4 ml. of 95% alcohol saturated with "triple acetate" and stir up precipitate with a fine glass rod. Rinse the rod into tube with 1 ml. of 95% alcohol solution. Centrifuge and drain completely. Add about 3 ml. of distilled water, insert a clean rubber stopper and shake vigorously until solution is complete. Transfer quantitatively with more water to a 250 ml. volumetric flask. Wash stopper with a few drops of distilled water before making up to mark. Add 1 drop of glacial acetic acid, 0·5 ml. of 20% potassium ferrocyanide solution and water to the 250 ml. mark. Mix and allow to stand for three minutes.

Compare in the colorimeter with 1 ml. of standard NaCl solution ($\equiv$ 0·7 mg. Na) which has been treated in parallel with 10 ml. of alcohol zinc uranyl solution and subsequently in the same way as the unknown.

Calculation. Let S and U mm. be the depth of the standard and unknown respectively. The standard contains 0·7 mg. Na. The unknown was prepared from the equivalent of 0·2 ml. of serum. Therefore Na in 0·2 ml. of serum equals $\frac{S}{U} \times 0{\cdot}7$ mg. and 100 ml. of serum contain $\frac{S \times 0{\cdot}7}{U} \times \frac{100}{0{\cdot}2}$ mg., *i.e.* serum sodium = $\frac{S}{U} \times 350$ mg. per 100 ml.

Check on Purity of Reagents. Take 0·5 ml. of the standard NaCl solution ($\equiv$ 0·35 mg. Na) and 1·0 ml. ($\equiv$ 0·7 mg. Na) and treat both as described above. Set the more concentrated at 20 mm. and match the more dilute against it. The reading of the latter should be 40 mm. If it is less than 40, one or more of the reagents contains Na as impurity.

Estimation of Blood Chlorides

Method of Van Slyke and Sendroy (17)

1 ml. of whole blood or plasma.

Reagents. 1. $\frac{N}{20}$ silver nitrate. Dissolve 0·495 g. $AgNO_3$ in

a minimum amount of water and dilute to 1 litre with nitric acid (sp. gr. 1·4).

2. $\frac{N}{50}$ ammonium thiocyanate.

3. 5% ferric alum in water.

Method. Introduce 1 ml. of plasma or blood into a 100 ml. hard glass test tube and add slowly and with constant shaking 3 ml. $\frac{N}{20}$ silver nitrate solution. Cover with a glass stopper and heat in a water bath until the solution above the silver chloride precipitate is clear and light yellow in colour. Add 6 ml. ferric alum solution, cool well in ice-cold water and titrate the excess silver with the standard thiocyanate until a definite salmon-pink colour is obtained.

Calculation. $117 \times (7{\cdot}5 - \text{ml. } \frac{N}{50} \text{ thiocyanate}) = \text{mg. NaCl}$ per 100 ml. blood. Control titrations are made in which 3 ml. of the silver nitrate solution, after adding 6 ml. ferric alum solution, are titrated directly with $\frac{N}{50}$ thiocyanate. If the latter varies from 7·5 ml., the calculation must be appropriately modified.

Estimation of Inorganic Phosphate

Method of Youngburg and Youngburg (18)

1 ml. of serum or plasma.

Reagents. 1. 10% trichloracetic acid.

2. Stock phosphate solution: Dissolve 0·4389 g. pure dry monopotassium phosphate in water and dilute to 1 litre (10 ml. = 1 mg. P). Preserve with a few drops of chloroform.

Standard phosphate solution: Dilute 10 ml. of the stock solution to 100 ml. 1 ml. = 0·01 mg. P.

3. Molybdic acid mixture. Mix 50 ml. of 7·5% sodium molybdate solution with 50 ml. of 10 N. sulphuric acid.

4. Reducing reagent: Stock: dissolve 10 g. stannous chloride in 25 ml. concentrated hydrochloric acid. Store in a brown glass stoppered bottle.

Standard or working solution: Dilute 1 ml. of the stock to 200 ml. with water. This should be freshly prepared each day.

Method. Transfer 4 ml. of 10% trichloracetic acid to a test tube, add 1 ml. of plasma or serum while shaking. Mix well and filter through a small phosphate-free filter paper. Transfer 2 ml. of filtrate to a test tube and 4 ml. of standard phosphate solution to a 6 in. by 1 in. test tube. Treat the solutions as follows:

Unknown	*Standard*
5 ml. water.	10 ml. water.
2 ml. molybdic acid.	2 ml. molybdic acid.
1 ml. dilute stannous chloride.	2 ml. stannous chloride.

The stannous chloride should be added rapidly *while rotating the liquid in the tube.* Compare in the colorimeter after five minutes, setting the standard at 20 mm.

Calculation. $\frac{100}{\text{Reading}}$ = mg. P per 100 ml.

N.B. The colorimeter cups and plungers must be perfectly free from phosphate (from blood sugars, etc.) and should be rinsed with distilled water several times before washing with the standard solution.

Estimation of Thiocyanates in Blood

Method of Ravin (19)

1 ml. of serum.

Reagents. 1. 20% trichloracetic acid solution.

2. Ferric nitrate solution. Dissolve 5 g. crystalline ferric nitrate ($Fe(NO_3)_3 6H_2O$) in 50 ml. of distilled water. Add 2·5 ml. of concentrated nitric acid and make up to 100 ml. with distilled water.

3. Standard solutions. Dissolve 0·057 g. of potassium dichromate and 3·83 g. of cobalt chloride ($CoCl_2\ 6H_2O$) in 100 ml. of distilled water This solution corresponds to a thiocyanate standard of 10 mg. per 100 ml. of serum. Lower standards, corresponding to 1 to 9 mg. per 100 ml. are prepared by appropriate dilution of this standard and all standards are sealed in comparator tubes (diam. $\frac{7}{16}$ in.). A blank of distilled water is essential.

Method. Pipette 2 ml. of water and 0·5 ml. of 20% trichloracetic acid into a centrifuge tube. Add 1 ml. of serum, mix

well and centrifuge. Transfer 1 ml. of the clear supernatant liquid into a comparator tube, add 0·2 ml. of the ferric nitrate solution and match the colour formed. The reading is in mg. of thiocyanate per 100 ml. of serum. Less than 1 ml. of serum may be used if there is insufficient material or if the reading is too high. Under these circumstances the calculation should be suitably modified according to the procedure adopted.

URINE ANALYSES

Estimation of Urea in Urine

Method of Folin and Youngburg (20) Modified by Barrett (4)

Reagents. The urease, Nessler's reagent and sodium hypochlorite are described under Blood Urea (see p. 373). The following are required in addition.

1. Standard ammonium sulphate solution. Dissolve 0·4402 g. of pure ammonium sulphate in water and dilute to 1 litre. Five ml. of this solution are equivalent to 1 mg. urea.

2. Phosphate buffer solution. Dissolve 14·2 g. of Na_2HPO_4 and 12·0 g. of NaH_2PO_4 in sufficient water to make 200 ml.

Method. Take the specific gravity of the urine. Dilute 1 ml. of urine with water from a burette to give a total volume in ml. equal to the last two figures of the specific gravity. For example, if the specific gravity is 1,020, dilute 1 ml. of urine to 20 ml. Add 1 g. of dust-free permutit for every 10 ml. of liquid, and shake the mixture for five minutes. Transfer 1 ml. of clear supernatant liquid to a clean test tube and add 0·5 ml. of urease solution and one drop of phosphate buffer. Incubate the tube at 55° C. for five minutes, then wash the contents of the tube into a 100 ml. flask, using a funnel. Prepare the Nessler-hypochlorite mixture by adding one drop of 10% sodium hypochlorite to each 10 ml. of Nessler's reagent used. Add 10 ml. of this mixture to the flask, which is twirled to mix the contents during the addition. Dilute to the 100 ml. mark with water, mix the contents of the flask and compare the solution in the colorimeter, setting the standard at 20 mm. and using a blue filter to facilitate matching.

Standard. Transfer 5 ml. of standard ammonium sulphate.

0·5 ml. urease, one drop of phosphate buffer and about 60 ml. of water to a 100 ml. flask. Add 10 ml. of Nessler-hypochlorite reagent and dilute to the mark.

Calculation. $\frac{2 \times \text{number of times urine is diluted}}{\text{Reading of unknown}}$ equals grams of urea per 100 ml. urine.

Estimation of Sulphonamides in Urine

Method of Gardner and Dunn (12)

Reagents. As described for blood on p. 385.

Method. The urine is diluted to contain 1 to 2 mg. of sulphonamide per 100 ml., and 50 ml. of the diluted urine together with 5 ml. of 4 N. hydrochloric acid are made up to 100 ml.; 10 ml. of this mixture are treated as for blood filtrate (see p. 385) to determine the free drug, and 10 ml. are heated without further addition for the determination of total sulphonamide.

Estimation of Vitamin C (l-Ascorbic Acid) in Urine

Method of Johnson and Zilva (21)

1 ml. of urine.

Reagents. 1. Standard l-ascorbic acid solution, 1 mg./ml. Weigh accurately 0·05 g. of pure l-ascorbic acid and dissolve in distilled water in a 50 ml. graduated flask. Acidify by adding 1 ml. of glacial acetic acid and a knife point of 8-hydroxy-quinoline to act as an anti-oxidant. Make up to the mark.

2. $\frac{1{\cdot}14\ \text{N}}{1{,}000}$ phenol-indo-2 : 6-dichloro-phenol. Weigh out 0·2 g. of solid phenol-indo-2 : 6-dichloro-phenol and dissolve in approximately 50 ml. of warm distilled water. Filter into a 100 ml. graduated flask; wash the filter paper and after cooling make up to the mark. Remove 10 ml. of this concentrated solution and dilute accurately ten times.

Pipette 1 ml. of the l-ascorbic acid solution into a conical flask, acidify with 1 ml. of glacial acetic acid and titrate against

the diluted indicator. According to this titration dilute the bulk of the original concentrated phenol-indo-2 : 6-dichloro-phenol solution to titrate 10 ml. for each milligram of l-ascorbic acid. Check the strength of the dilute solution by titration against the standard l-ascorbic acid solution. One ml. of l-ascorbic acid $\equiv$ 10·0 ml. $\frac{1{\cdot}14\ N}{1{,}000}$ phenol-indo-2 : 6-dichloro-phenol.

Principle. The redox indicator phenol-indo-2 : 6-dichloro-phenol is reduced by l-ascorbic acid in acid solution to its leuco-base. The colour change of the dye is utilised to indicate the end of the titration reaction.

Method. Pipette out 1 ml. of urine sample. Acidify with 2 to 3 drops of acetic acid. Run in $\frac{1{\cdot}14\ N}{1{,}000}$ phenol-indo-2 : 6-dichloro-phenol from a burette until a permanent pink appears. If the titration is under 1 ml., repeat using 5 ml. or 10 ml. of urine as deemed necessary. When heavily pigmented urines are used, compare with an aliquot volume of urine as a blank.

Calculation. The titration figure, divided by ten gives the amount of l-ascorbic acid in mg. present in the volume titrated.

Estimation of Neutral 17-Ketosteroids

Method of Talbot, Wolfe, MacLachlan and Berman (22)

Twenty-four-hour urine sample (toluene preservative).

Reagents. 1. Sodium hydroxide solutions : (*a*) N solution, (*b*) 2·5 N solution.

2. Sodium hydrosulphite solution. 10% sodium hydrosulphite made up in N sodium hydroxide.

3. $\frac{N}{2}$ hydrochloric acid.

4. Girard T reagent (solid). Trimethyl-ammonium-aceto-hydrazide-chloride.

5. Absolute ethyl alcohol. Aldehyde content must be lower than 0·0025%.

6. Diethyl ether—should be free of peroxides—AR quality.

7. Benzene (crystallisable).

8. 2·5 N potassium hydroxide in absolute ethyl alcohol solution. The solution may be made fresh daily by dissolving 1·4 g. AR potash in 10 ml. of absolute alcohol with mechanical shaking. Filter through hardened filter paper (Whatman No. 50). The solution which should be crystal clear may be stored up to five days at 0° C.

9. Meta-dinitrobenzene. This material should have a very high degree of purity. The compound as supplied should be further purified as follows: 20 g. are dissolved in 750 ml. of 95% alcohol and warmed to 40° C. One hundred ml. of 2 N sodium hydroxide are added. After five minutes the solution is cooled and 2,500 ml. of water are added. The precipitated m-dinitrobenzene is collected on a Buchner funnel, washed thoroughly with water, sucked dry and recrystallised twice in succession from 120 ml. and 80 ml. of absolute alcohol by dissolving in the quantity of alcohol stated and evaporating off alcohol until crystallisation commences. The purified material must be well crystallised, almost colourless needles of melting point 90·5 to 91° C. A mixture of a 1% alcoholic solution of the compound with an equal volume of aqueous 2 N NaOH should give no colour after an hour.

The reagent is used as a 2·5% solution of purified m-dinitrobenzene in absolute alcohol. This solution may be stored in a dark bottle at 0° C. for approximately one month, but each time before use the excess solid should be brought into solution by gentle warming.

Method. 1. Hydrolysis and extraction. Add to the twenty-four-hour urine sample 150 ml. of concentrated hydrochloric acid per 1,000 ml. of urine and hydrolyse by heating under reflex for thirty minutes. Measure out 200 ml. or some convenient aliquot and extract with 50 ml. of benzene for sixteen hours. Distil off the benzene and take up the residue in 25 ml. of ether. An alternative process for combined hydrolysis and extraction is described by Callow et al. (23).

2. Preparation of neutral 17-ketosteroid fraction. Transfer the ether solution to a separating funnel and wash consecutively with (i) three lots of 5 ml. of sodium hydrosulphite solution, (ii) twice with 5 ml. of N sodium hydroxide solution, (iii) twice

with 5 ml. of $\frac{N}{2}$ hydrochloric acid and finally (iv) three times with 10 ml. of water. The ether solution is then separated off and heated to dryness on a water bath. The neutral gummy residue is dissolved in 0·10 ml. of glacial acetic acid and 40 mg. of Girard T reagent are added. The reaction, which is carried out in a ground glass-stoppered flask, is promoted by heating for sixteen minutes. Allow to cool and add 10 ml. of iced water and 0·75 ml. of 2·5 N sodium hydroxide. Extract four times with 10 ml. of ether and, after washing this ether three times with 10 ml. of water, reject the ether solution. To the aqueous extract plus washings add 0·2 ml. of concentrated sulphuric acid and 4 ml. of ether. Allow to stand at least two hours, but preferably overnight. Add 0·2 ml. of concentrated sulphuric acid and extract four times with 10 ml. of ether. Wash this ether extract three times with 5 ml. of water. Separate and dry the ether solution with anhydrous sodium sulphite. Filter, evaporate to dryness and dissolve the residue in 1 ml. of absolute alcohol.

3. Colorimetric estimation. Select three glass-stoppered tubes of approximately 15 ml. capacity and pipette in successively 0·2 ml. of alcoholic solution, 0·1 ml. of alcoholic solution, plus 0·1 ml. of alcohol and 0·2 ml. of alcohol. These tubes represent respectively (i) a high concentration test, (ii) a low concentration test, and (iii) a blank. Into each tube pipette 0·2 ml. of 2·5% alcoholic m-dinitrobenzene solution and 0·2 ml. of 2·5 N alcoholic potassium hydroxide. Mix and place the tubes in a water bath at 25° C. for one hour. Add 10 ml. of absolute alcohol to each tube. Mix and read in a colorimeter or absorptiometer within five minutes of dilution.

4. Calibration. For colour comparison a colorimeter or absorptiometer may be employed. In the former case comparisons are made against a $\frac{N}{1{,}000}$ potassium permanganate solution, whilst for the latter instrument pure androsterone or the less active but more readily accessible iso-dehydro-androsterone may be employed as suitable reference compounds. Callow, Callow and Emmens (24) recommend the use of a filter transmitting light in the region of 5,500 Å (Ilford Spectrum yellow-

green filter). Suitable standard solutions for calibration purposes range from zero to 0·4 mg. of androsterone per 0·2 ml.

Differential Estimation of 3α and 3β Neutral 17-Ketosteroids

Method of Langstroth, Talbot and Fineman (25)

Carried out on neutral 17-ketosteroid fraction (p. 397).

Reagents. 1. Benzene, absolute alcohol, A.R. ether, m-dinitrobenzene, and 2·5 N KOH in absolute alcohol as for estimation of total ketosteroids.

2. Aqueous digitonin solution (7 mg. digitonin/ml.). This solution is prepared by making up an aqueous digitonin solution containing 1 mg. per ml. This is allowed to stand overnight, and is then filtered and concentrated to 1/7 volume by evaporation in a boiling water bath.

Principle. Separation of 3α and 3β ketosteroids depends on the fact that 3β-ketosteroids are precipitated with digitonin as an equimolecular complex of sterol and digitonin, whereas 3α-ketosteroids do not react. The estimations, which are carried out colorimetrically, involve the following procedures :—

(*a*) Determination of total 17-ketosteroids in the final alcoholic solution.

(*b*) Precipitation of β-ketosteroids in an aliquot of the solution by means of digitonin.

(*c*) Removal of the precipitate.

(*d*) Purification of the supernatant fluid which contains the α-ketosteroids.

(*e*) Determination of the α-ketosteroids in an alcoholic extract of the supernatant fluid. The β-ketosteroids are determined by difference.

Method. 1. Precipitation of β-ketosteroids. 0·5 ml. acetone, 2·0 ml. digitonin solution and 1 ml. of the final alcoholic solution of ketosteroids are placed in a small flask fitted with a ground-in glass stopper, shaken carefully, and allowed to stand overnight with the stopper inserted in the flask, which is preferably kept in an atmosphere of alcohol vapour to prevent evaporation.

Next day, the fluid in the flask is transferred to a clean dry centrifuge tube, and spun at 2,000 r.p.m. for fifteen minutes.

A measured amount (*e.g.* 2 ml.) of the supernatant fluid is transferred to a clean dry glass-stoppered flask.

N.B. The precipitate is an equimolecular complex of sterol and digitonin. The molecular weight of digitonin is four times that of dehydroisoandrosterone, so that the 14 mg. of digitonin added should be capable of precipitating more than 2 mg. of dehydroisoandrosterone, *i.e.* the procedure described above should be adequate for concentrations of 3β-ketosteroids up to at least 2 mg. per ml. of alcoholic solution. In cases of suspected adrenal cortical tumour with high total ketosteroid excretion, of which a high proportion is probably β-ketosteroid, 0·5 ml. or 0·25 ml. of alcoholic solution of ketosteroids should be taken for precipitation of β-ketosteroids, the volume being made up to 1 ml. with absolute alcohol.

2. Purification of the supernatant fluid. To the aliquot (*e.g.* 2 ml.) of supernatant fluid in the stoppered flask, 25 ml. of benzene are added, and the mixture shaken vigorously for five minutes. The mixture is allowed to stand for several minutes to allow of partial separation of the aqueous and benzene phases, and as much of the benzene as can be decanted without including any of the aqueous phase is then filtered through a soft filter paper (*e.g.* Whatman No. 1) into a small dry flask.

A measured amount of this benzene solution (usually 20 ml.) is transferred to a small distillation flask and the benzene is distilled off under reduced pressure.

To the dry residue in the distillation flask, 20 ml. of ether are added and the ether distilled off at atmospheric pressure. Another 20 ml. ether are added to the flask and distilled off as before.

The dry residue is taken up in 1 ml. absolute alcohol, and the α-ketosteroid content estimated on 0·2 ml. and 0·1 ml. aliquots colorimetrically as described under the routine procedure for total 17-ketosteroids. When calculating the amount of α-ketosteroid present in the original alcoholic solution of total ketosteroids, allowance must be made for the fact that aliquots only were taken at two stages—after precipitation of β-ketosteroids and after extraction of α-ketosteroids with benzene.

The purification procedure described above is necessary because some of the added substances interfere with the Zimmermann reaction. The supernatant fluid, after precipitation of β-keto-

steroids, contains water, acetone and digitonin as well as the dissolved α-ketosteroids. The water and digitonin are eliminated by extraction of the α-ketosteroids with benzene, while the acetone and benzene are eliminated by evaporation.

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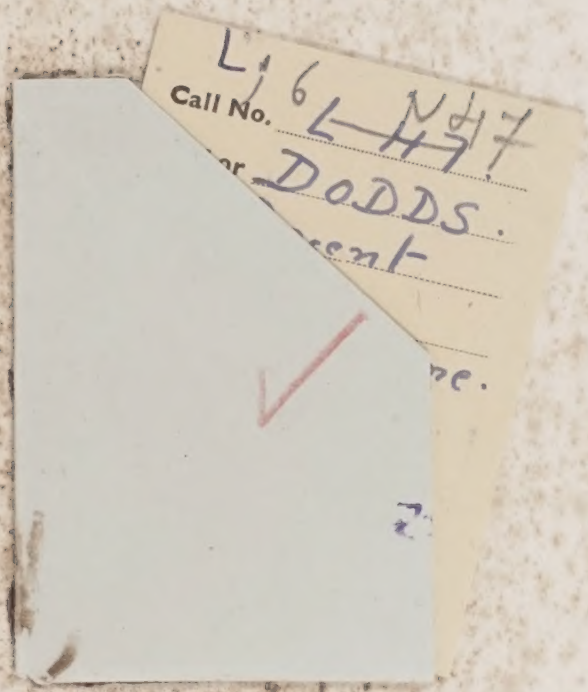
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